LINSEED MUCILAGE.

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THE vegetable mucilages have been, as a whole, but little investigated, probably owing to the difficulties attending their preparation in anything like a state of chemical purity. Workers who have turned their attention to these substances have generally contented themselves with a simple chemical investigation and have left practically untouched the question of the fate of these substances in the animal organism. Thus, amongst other workers, Harlay[1] investigated the mucilage of prickly pear, v. Bittó[2], the mucilage of capsicum seed, Gaus and Tollens[3] quince mucilage, Yoshimura[4], the mucilages from several plants, while Hilger[5] examined the mucilage of linseed. With the exception of the latter the work is frequently merely the identification of one or more sugars produced on hydrolysis, with, perhaps, a determination of pentose and hexose sugar-producing complexes. One is struck by the great variation reported between different mucilages. Not only do the sugars produced vary both in nature and quantity, but both the presence and absence of products of hydrolysis other than sugars are reported, while in some cases the original compound is stated to be capable of combining with bases and in other cases to be neutral in character. Probably the compounds, which have been designated mucilages, should not be included in one chemical class, for they have been more frequently characterised by the physical properties of their solutions than by their chemical constitution. Cross and Bevan, however, in their work on "Cellulose," make two definite classes, one, the true mucilages, or muco-celluloses, which yield nothing but sugars on hydrolysis, the other, the pecto-celluloses, which give acid hydrolysis products as well

[1] *J. Pharm. Chim.*, 1902 [vi], 16, 193—198.
[2] *Landw. Versuchs-Stat.*, 1895, 46, 1309.
[3] *Ann.*, 249, 245.
[4] *Bull. Coll. Agric.*, Tokyo, 1895, 2.
[5] *Ber.*, 1903, 36, 3197—3203.

as sugars. In the present state of knowledge of the mucilages it is difficult to say whether such a classification will finally hold or not, for, owing to the difficulties in purifying mucilaginous substances, it is almost impossible to say whether the substance dealt with is a single chemical individual or a mixture. But in a case like linseed mucilage, where exceedingly large quantities are consumed every year by cattle and sheep, whether the substance, which, when purified certainly behaves as though it contains a very greatly preponderating quantity of one chemical individual, be a single substance or not, a knowledge of its chemical composition and fate in the animal organism will certainly be valuable, and the present work was undertaken from that point of view.

Bauer[1] had already identified dextrose in the products of hydrolysis. Kirchner and Tollens[2] state that they obtained arabic acid and cellulose on hydrolysis, while Hilger[3] investigated the substance much more fully and the main facts worked out by him were confirmed and are noted in the text.

The work naturally divided itself into the purely chemical investigation, and the examination of the substance as a feeding stuff; and in that order the experimental work is given below.

Preparation of the Mucilage.

Two methods for the extraction of mucilage from the seed were tried; extraction by 1 per cent. sulphuric acid in the cold and extraction with cold water only. The solvent was, in each case, allowed to act on the linseed for 24 hours, when the mucilaginous liquid was squeezed away from the seed through fine linen, and the process then repeated until very little mucilage could be obtained from the extract. The extracts so obtained contained a small amount of flocculent matter which was allowed to settle and the mucilage solution decanted therefrom, filtration being so slow as to be impracticable. Both extracts were then treated with phosphotungstic acid solution (2·5 grs. phosphotungstic acid and 5 grs. sulphuric acid in 100 c.c. water) until no further precipitate was obtained. The water extract gave only a slight precipitate with the reagent, while the acid extract gave a voluminous precipitate. As this indicated that the extraction of proteid matter was greater in the case of the acid solution, this method was discontinued and the water extract alone used.

[1] *Landw. Versuchs-Stat.*, 1892, 40, 480. [2] *Ann.* 175, 205.
[3] *Ber. loc. cit.*

The mucilage solution was now treated with twice its bulk of 90 per cent. alcohol, when the mucilage was obtained as a white fibrous mass, which was dehydrated with absolute alcohol, washed with ether and finally dried in vacuo. The substance so prepared contained 1·5 per cent. ash and 1·8 per cent. proteid matter, the latter being calculated from a Kjeldahl nitrogen determination. The ash content was further decreased by treating the substance with very dilute hydrochloric acid and again precipitating and drying, when the ash content was reduced to 0·6—0·7 per cent. Attempts at further purification by again acidifying with hydrochloric acid and dialysing did not give results which justified the use of the process on a larger scale. The quantity of mucilage obtained by this method was much smaller than anticipated. In one case 700 grs. of linseed when exhaustively extracted gave 44 grs. of mucilage or 6·28 per cent. Other extractions gave figures agreeing closely with this figure.

PROPERTIES OF THE MUCILAGE.

Prepared in the manner described above the mucilage is obtained as a friable white powder which "dissolves" in water to an opalescent "solution" which while neutral to litmus is capable of neutralising caustic alkalies. In an actual experiment 0·334 gm. mucilage required 0·0188 gm. sodium hydroxide for neutralisation to phenol-phthalein, the estimation being made by adding excess of the alkali and titrating back with acid. If the substance is assumed to behave as a monobasic acid this result would give a molecular weight of 710. From the general properties of the substance however this is, without doubt, only a sub-multiple of the true molecular weight. It may be noted that it agrees closely with a molecule four times the size of $C_6H_{12}O_6$ which is the empirical formula for the substance given by the combustion results described below. The solutions in caustic alkalies are quite clear and the substance appears to be in true solution. . These solutions are dextrorotatory and a determination of rotatory power shewed that 0·2085 gm. mucilage, exactly neutralised with caustic soda, and made up to 25 c.c. with water, gave $a = +0·09$ in a 1 dcm. tube,

whence $$[a]_D = +10·79.$$

Clear solutions may also be obtained in ammonia but in this case considerable excess of the reagent is necessary. With alkali carbonates the mucilage solutions give no reaction.

The substance is slowly hydrolysed by boiling with dilute mineral acids. It is very slowly, if at all, attacked by hot alkali. It gives no coloration with iodine, does not reduce Fehling's solution, and gives no reaction with phenylhydrazine.

Solutions of the salts of the heavy metals give gelatinous precipitates, and the substance itself is insoluble in all the organic solvents.

As extracted from the seed, the mucilage contains a considerable quantity, about 2 per cent., of ash and this contains calcium, potassium, magnesium, iron and phosphoric acid.

The mucilage does not melt below 250° C. and is apparently unchanged by heating for some time at 150° C. though when heating above 200° C. is prolonged for some time it gradually becomes brown in colour and some decomposition takes place.

On combustion it gave the following result:

0·1789 gm. substance gave 0·2753 gm. CO_2 and 0·1030 gm. H_2O,

whence C = 41·96 per cent.; H = 6·37 per cent.

$(C_6H_{12}O_6)_n$ requires C = 40 per cent., H = 6·66 per cent.,

Although in the carbon estimation there is nearly 2 per cent. difference between the estimation and the empirical formula suggested, it must be remembered that it is impossible, owing to the nature of the substance, to obtain it chemically pure for analysis: the figures given however agree more closely with the suggested formula than with any other carbohydrate formula.

In a Berthelot bomb calorimeter (of water equivalent 333) 0·7642 gm. mucilage, with 0·0145 gm. iron firing wire, gave a rise of 1·298° C. to 2000 c.c. water. The radiation correction was − 0·0024° C.

From the above, the heat of combustion for 1 gm. of the substance is shown to be 3925 calories. This is of the order to be expected from a body of carbohydrate nature and compares with 3866 for cane sugar, 4123 for starch and 4146 for cellulose.

Nitration with concentrated nitric acid was unsuccessful, but the hydrated cellulose nature of the mucilage was very definitely shown by the action of acetyl chloride and benzoyl chloride.

Acetyl Derivative. The substance was boiled with excess of glacial acetic anhydride for several hours. The action was very slow but after some time the substance dissolved completely, when the solution was filtered, poured into cold water and well shaken. A white flocculent precipitate was obtained, and this was purified by redissolving in glacial

acetic acid and precipitating by water or ether. The substance so obtained was a white amorphous body, soluble in glacial acetic acid, ethyl acetate and acetone, moderately soluble in a mixture of alcohol and ether, and insoluble in other solvents. Films of the solutions dry down to transparent glassy masses.

The substance melted at 240°—245° C., with decomposition, and on combustion gave the following figures:

0·1639 gm. gave 0·2837 gm. CO_2 and 0·0834 gm. H_2O,

whence C = 47·20 per cent., H = 5·65 per cent.,

$C_6H_9O_6(CO{\cdot}CH_3)_3$ requires C = 47·06 per cent., H = 5·88 per cent.

Benzoyl Derivative. The substance was dissolved in caustic soda solution (actual NaOH = 20 per cent. of weight of mucilage) and benzoyl chloride added gradually, with constant shaking, until a large excess of the reagent had been used. A flocculent white precipitate was formed, which was filtered off, washed and dried. The substance was thus obtained as a friable white powder melting with decomposition at about 270° C. It was insoluble in water and ether, slightly soluble in acetic acid, and to a still slighter degree in alcohol.

0·1327 gm. gave 0·2992 gm. CO_2 and 0·0620 gm. H_2O,

C = 61·49 per cent.; H = 5·18 per cent.,

$C_6H_{10}O_6(CO{\cdot}C_6H_5)_2$ requires C = 61·85 per cent., H = 5·16 per cent.

The formation of these two derivatives shows that the mucilage is very probably a hydrated cellulose, since the typical cellulose itself behaves in a similar manner with acetyl and benzoyl chlorides, and shows thereby the presence of hydroxyl groups, two of which are situated differently from the third, in the simple molecular unit.

Although from its general behaviour as a colloid it was anticipated that no molecular weight determinations on the mucilage would be possible, the attempt was made with Walker and Lumsden's modification of Landberger's boiling point apparatus. The results were only negative, the minute changes of boiling point noticed being easily accounted for by the small ash content of the substance.

HYDROLYSIS OF THE MUCILAGE.

When boiled for some hours with dilute mineral acids the mucilage is completely hydrolysed, though the process is slower than was anticipated. Dilute sulphuric acid (4 per cent.) was used, and the dark brown

solution obtained after six hours boiling was filtered free from a small quantity of flocculent matter, treated with animal charcoal, filtered and baryta added until just alkaline. The barium sulphate was filtered off and to the clear solution alcohol added in considerable quantity. There was thus precipitated a small quantity of amorphous white substance containing a considerable quantity of barium which was filtered off from a clear yellowish solution. The alcohol was distilled off from the solution and the three products (*a*) the flocculent matter obtained on first hydrolysing, (*b*) the solid precipitated by alcohol, and (*c*) the clear aqueous solution, examined separately.

(*a*) This product is small in quantity, is of a humic nature and very probably not a hydrolysis product of the mucilage itself since, with the purest samples of mucilage used, the quantity formed was practically negligible, while with cruder samples much larger quantities were obtained. The author has little doubt that this product would be entirely absent if it were possible to work with chemically pure mucilage.

(*b*) This substance appeared at first to be the barium salt of an organic acid. It was dissolved in water, sulphuric acid added till all the barium was precipitated and the barium sulphate filtered off. The solution remaining was evaporated to small bulk and the last traces of water taken off in a vacuum desiccator. The material so obtained was found to contain phosphoric acid. This was removed as calcium phosphate and the other acid obtained in the form of its calcium salt. This substance on ignition gave in two cases 16·62 per cent. and 16·93 per cent. CaO.

The calcium salt is amorphous, easily soluble in water and strongly dextrorotatory.

0·249 made up to 25 c.c. with water gave $\alpha = +1·3$ in a 2 dcm. tube, whence $[\alpha]_D = +65·26$.

On combustion of a specimen of this calcium salt the following results were obtained:

0·1704 gm. gave 0·2032 gm. CO_2 and 0·0986 gm. H_2O,

C = 32·51 per cent., H = 6·43 per cent.

The substance contains no nitrogen, does not reduce Fehling's solution, and the free acid dissolved in sodium carbonate solution does not decolorise permanganate solution. Also it does not give, so far as could be detected by the ordinary colour reaction, any furfurol on treatment with hydrochloric acid.

The barium and cadmium salts which were also prepared are amorphous and present no advantage for manipulation over the calcium salt. The quantity of this acid product of hydrolysis varies slightly with different samples of mucilage, but more than 2·5 per cent. was never obtained, and this small yield renders the investigation of the substance somewhat difficult.

It may be noted here that, whatever be the constitution of this acid body, it can hardly be, as stated by Kirchner and Tollens[1], the ordinary form of arabic acid, since this acid is laevorotatory, while the mucilage acid is strongly dextrorotatory. In a general way, the mucilage acid appears to be similar to the geddic acid described by O'Sullivan[2], and may be produced from a complex carbohydrate in a similar manner to geddic acid from gedda gum.

The author is not convinced that the acid is necessarily a hydrolysis product of the mucilage since the method of extraction of the mucilage does not preclude the presence of other non-nitrogenous bodies in the aqueous extract, and the small and varying quantity which is obtained of this product lends some support to this view.

(*c*) The aqueous solution has a strong reducing action on Fehling's solution and in a preliminary experiment it was shown that this aqueous solution from the hydrolysis of a known weight of mucilage had practically the same cupric reducing power as an equal weight of hydrolysed starch. The solution was investigated as follows:

(1) To a portion of the solution strong nitric acid was added until the specific gravity reached 1·3, and the mixture was then heated for some time at 60° C. The liquid was then diluted with water and allowed to stand, when a white crystalline precipitate separated out. This was extracted with hot alcohol to remove any oxalic acid present and the residue was recrystallised from hot water. It was thus obtained as a white crystalline powder melting at 225° C. with decomposition, and giving results on analysis corresponding with mucic acid. The substance was therefore mucic acid and the original solution contained galactose.

(2) A portion was oxidised with bromine water at the ordinary temperature, the excess of bromine removed by heating, the solution neutralised by cadmium carbonate, evaporated down, and alcohol added. A white solid crystallising in prismatic needles was obtained. The substance had no definite melting point but an estimation of bromine corresponded with the composition $C_5H_9O_6 . CdBr . H_2O$, the double

[1] *Ann. loc. cit.* [2] *J. C. S.*, 1891, T. 1029.

cadmium salt of xylonic and hydrobromic acids, and the formation of this was shown by Bertrand[1] to be characteristic of xylose.

(3) A large portion of solution was treated with phenylhydrazine, and the mixed osazones which separated were collected in two portions, the first being a yellow solid substance and the second a dark brown oil which afterwards solidified. By fractional crystallisation of these from dilute alcohol there were separated the osazones of glucose (M.P. 204° C.), arabinose (M.P. 160° C.) and xylose (M.P. 150—151° C.).

(4) A portion of the solution was treated with diphenylhydrazine and the precipitate produced was purified several times by crystallisation. It showed the characteristics of xylose diphenylhydrazone and melted at 204—205° C.

There were thus identified in the solution the four sugars glucose, galactose, arabinose and xylose which Hilger[2] had noticed.

Experiments were now carried out with a view to obtaining some quantitative determinations of the sugars obtained by hydrolysis. Ten grams of mucilage, containing, when allowance had been made for the water, ash and protein content, 8·2 gm. pure mucilage were hydrolysed with 4 per cent. sulphuric acid. The proteid matter was removed by phosphotungstic acid, the mineral acid precipitated by baryta, and the barium salt of the acid decomposition product precipitated by alcohol. The solution which should now contain sugars only was evaporated to small bulk and the remaining water removed *in vacuo*. There were thus obtained 7·31 gm. of a yellowish solid mass, which was practically ash free, quite nitrogen free, and which, as far as could be shown by qualitative tests, contained sugars only. That is, 89·1 per cent. of the weight of the mucilage taken can be recovered as sugars.

The sugars were then distilled with hydrochloric acid, as for the usual estimation of pentosans, and the furfurol distilling over was precipitated as the phenylhydrazone. There was thus obtained hydrazone corresponding to 1·1463 gm. pentose sugar, $C_5H_8O_4$. This corresponds to 16·88 per cent. of the total sugars existing as pentoses.

Another furfurol determination done directly on 8·204 gm. pure mucilage (when allowance had been made for water, ash and proteid) gave hydrazone corresponding to 1·407 gm. pentose sugar or 17·15 per cent. of the pure mucilage, and this figure confirms the estimation above on the previously hydrolysed material.

[1] *Bull. Soc. Chim.* [3], 7, 499—502. [2] *Ber. loc. cit.*

The figures above would seem to indicate that practically the whole of the mucilage is hydrolysed to sugars, and that of the sugars about 17 per cent. are pentoses. Since two pentose sugars were identified, an expression such as $2\,C_5H_8O_4 . 6\,C_6H_{12}O_6$ would seem to be the simplest way of representing the original constitution of the hydrolysis mixture, since this expression requires 19·6 per cent. pentose sugars, and two of these sugars are present. This differs from the sample of mucilage investigated by Hilger, who found equal quantities of hexose and pentose sugars.

SUMMARY OF CHEMICAL INVESTIGATION.

The preceding experiments show that linseed mucilage is a carbohydrate body showing all the characteristics of the hydrated cellulose, and that it is well described by the term "muco-cellulose" under which such substances are classed by Cross and Bevan. It gives on hydrolysis both hexose and pentose sugars in such quantity that, for practical purposes, at any rate, it can be considered to give nothing else. In fact it is very doubtful whether the other products obtained in hydrolysing an average sample are decomposition products or, at any rate, direct, decomposition products of pure mucilage. The author inclines to the opinion that they are not.

ACTION OF ENZYMES ON MUCILAGE.

From a practical point of view the possible fate of the mucilage in the animal organism is of considerable importance, and the action of the different digestive ferments on the material was investigated.

In most cases 5 gm. of mucilage were weighed out, dissolved in a considerable quantity of water, the enzyme preparation added and the whole made up to 250 c.c. with water, or smaller quantities were used at the same concentration. The solution was made faintly acid or alkaline as required by the different ferments, and any sugar formed was estimated indirectly by its cupric reducing power, using ferric alum and standard permanganate solution (1 c.c. of the latter being equivalent to ·005 gm. dextrose) to estimate the Cu_2O produced. In the estimations the unchanged mucilage was precipitated by alcohol, filtered off, well washed, dried and weighed. The filtrate, which should contain any sugar formed, was freed from alcohol by heating and the sugar estimated as mentioned above. The reaction mixtures were kept at 41° C. in a thermostat, and in all cases the activity of the

enzyme preparation was tested by trying it on starch under exactly similar conditions.

The ferments were found to be without action on the mucilage solutions, a small initial reducing power of the solution remaining unchanged after many hours. Particulars are given below of some of the experiments, showing the general character of the results obtained.

Taka Diastase.

Two grams mucilage in 100 c.c. water. Ten c.c. taken for each estimation.

Sample	Time in hours	Permanganate required to oxidise Cu_2O formed	Grams sugar present calculated as dextrose	Mucilage recovered
1	0·75	2·3 c.c.	0·0115	0·1660 gm.
2	1·25	2·6 ,,	0·0130	0·1686 ,,
3	4·0	2·5 ,,	0·0125	0·1775 ,,
4	6·86	2·6 ,,	0·0130	0·1762 ,,
5	23·25	2·7 ,,	0·0135	0·1689 ,,
6	192·0	2·7 ,,	0·0135	0·1654 ,,

The weight recovered should be, of course, 0·2 gm., but the error in recovering and drying is considerable, and lack of increase in the reducing power of the solution shows that no reaction was taking place.

Barley Diastase.

Two grams mucilage in 100 c.c. water. Ten c.c. taken at a time for estimation.

Sample	Time in hours	Permanganate required to oxidise Cu_2O formed	Grams sugar present calculated as dextrose	Mucilage recovered
1	0·33	3·2 c.c.	0·0160	0·1727 gm.
2	0·66	3·0 ,,	0·0150	0·1686 ,,
3	1·9	3·4 ,,	0·0170	0·1630 ,,
4	7·0	3·1 ,,	0·0155	0·1640 ,,
5	26·0	2·7 ,,	0·0135	0·1685 ,,
6	53·2	2·4 ,,	0·0120	0·1605 ,,

"ZYMINE."

A commercial preparation of the "digestive principles of the pancreas." Five grams mucilage in 250 c.c. water. Twenty c.c. taken as test portion.

Sample	Time in hours	Permanganate required to oxidise Cu_2O formed	Grams sugar present calculated as dextrose	Mucilage recovered
1	0·33	2·7 c.c.	0·0135	0·3879 gm.
2	1·16	3·0 ,,	0·0150	0·3500 ,,
3	1·93	2·9 ,,	0·0145	0·3600 ,,
4	2·5	3·4 ,,	0·0170	0·3821 ,,
5	26·25	3·3 ,,	0·0165	0·3450 ,,

SALIVA.

Five grams mucilage treated with saliva made up to 250 c.c. with water. Twenty c.c. taken for each test portion.

Sample	Time in hours	Permanganate required to oxidise Cu_2O formed	Grams sugar present calculated as dextrose	Mucilage recovered
1	1·0	2·0 c.c.	0·0100	0·3620 gm.
2	3·25	2·3 ,,	0·0115	0·3657 ,,
3	5·25	2·2 ,,	0·0110	0·3598 ,,
4	25·0	2·2 ,,	0·0110	0·3421 ,,

PEPSIN.

Exactly similar results to the above were obtained with pepsin on the mucilage, although control experiments showed the enzyme preparation to be of normal activity. Even after thirty hours 89—90 per cent. of the mucilage could be recovered unchanged.

EXTRACT OF OX PANCREAS.

The same negative results were obtained with an extract of ox pancreas prepared in the laboratory.

FEEDING EXPERIMENTS.

The only conclusion to be drawn from the above results was that the common digestive enzymes were without action on linseed mucilage,

and presumably therefore it would pass through the animal, at any rate through the non-ruminants, unchanged. Feeding experiments were therefore carried out with animals.

The first experiments were attempted with guinea-pigs, the animals being fed for one period on green food and afterwards on the same food mixed with linseed. The animals proved unsuitable for the purpose. They did not readily eat the linseed, and it was difficult to devise any method for showing whether or not the mucilage was passing through the animal.

Rats proved, however, to be much more suitable, and an experiment was arranged as follows. Six rats, equally divided between three cages, were kept for a five or six day period on a prepared diet, and were afterwards fed for a similar period on the same diet, except that a certain amount of starch was replaced by prepared mucilage. The carefully weighed-out food was given in such quantity that each rat received about 9 gm. of dry matter per day, and the faeces were collected, dried and weighed. The loss of food and faeces must have been very small, as the open-work wire bottoms of the cages, which were raised above a sheet of white filter paper resting on sawdust, allowed all uneaten food and faeces to be collected easily and accurately, and, as the animals were only allowed a small quantity of cotton wool as bedding, loss therein was negligible. This arrangement of cages also prevented any appreciable admixture of the urine with the faeces, the former being taken up rapidly by filter paper and sawdust.

The tables below give the figures for the experiment and such particulars as are necessary for the working out of the final result.

Period I. Diet (A)		Period II. Diet (B)	
Starch	162·4 gm.	Mucilage	89·5 gm.
Sugar	81·2 ,,	Starch	81·2 ,,
Lard	48·8 ,,	Sugar	81·2 ,,
Casein	97·6 ,,	Lard	48·8 ,,
Ash	10·8 ,,	Casein	95·1 ,,
		Ash	5·0 ,,

(The "mucilage" used in diet (B) contained 5·8 gm. of ash and 2·5 gm. albuminoid matter, so that the second diet was identical with the first except for the replacement of 81·2 gm. starch by an equal quantity of pure mucilage.)

The ingredients were thoroughly rubbed together, made into small cakes with the help of a little water and then heated for a short time in a water oven. After this preparation, cakes from (A) contained 27·22 per cent. of water, while cakes from (B) contained 31·2 per cent. water.

In Period I, Cage I rats were, owing to an accident, only allowed a five-day period, but in all other cases a six-day period was given. The faeces were first collected 18 hours after the first meal was given and up to the same length of time after the last meal. An interval of a week on ordinary diet was given between the two periods.

Cage	Period I.		Period II.	
	Dry matter eaten	Faeces	Dry matter eaten	Faeces
	gm.	gm.	gm.	gm.
I	84·98	4·745	81·02	13·5
II	100·99	5·071	74·64	12·91
III	104·99	5·055	73·45	10·73

HEAT OF COMBUSTION OF FOOD AND FAECES.

Small calories per gram of dry matter.

Period I.		Period II.	
Food	Faeces	Food	Faeces
4960	4494	4785	4846

The heat of combustion of the sample of "mucilage" used was 3725 calories per gram.

From the above tables the following statement can be made out, taking the average value per rat per day.

Values per rat per day.

	Period I.	Period II.
Calories in food eaten ...	42408	30452
,, faeces ...	1966	5001
Per cent. of energy in faeces	4·64	16·42

The difference in the energy lost in the faeces in the two periods is 11·78 per cent. From the figures given it can be calculated that the mucilage provided 15·6 per cent. of the energy of the food.

It is difficult to suggest the probable experimental error in an experiment such as this, but the figures point to very slight utilisation of the mucilage by the animal. It can be stated with certainty that 75 per cent. of the mucilage passes through the animal unchanged, and

even the appearance of the faeces showed the presence of a considerable quantity of unchanged mucilage. It was still, however, possible that the linseed itself carried an enzyme which would cause the break-up of the mucilage when the latter was eaten in the ordinary way as part of the seed, and that the enzyme was absent in the diet given in the experiment above. A preliminary experiment on this point had already been done when carrying out the thermostat experiments with the digestive enzymes, a solution of the mucilage being treated with cold water extracts of both germinating and resting seeds. No action could be detected, but it was just possible that the seed contained an enzyme which only became active in the alimentary canal of the animal, as in the case recorded by H. Brown[1].

To test this point, a sample of linseed, containing 5·4 per cent. water, 24·25 per cent. proteid and 37·50 per cent. fat, was finely ground and made into small cakes with starch, sugar and casein in the following proportions :—

Linseed	220 gms.
Starch	200 „
Sugar	200 „
Casein	120 „

The food thus prepared was divided into two portions, one dried at the ordinary temperature and one heated to 110° C. for 15 hours in order to destroy any enzyme present. The quantity of food given and the faeces collected are given in the following table, the weights being the average ones per rat per day.

	Dry matter eaten	Dry matter in faeces	Calorific value of food	Calorific value of faeces
Heated food	7·886	0·828	4817 cal. per gm.	4909 cal. per gm.
Unheated food	8·312	0·911	4694 „ „	4666 „ „

From the figures given above the following comparison of the two periods is obtained.

Calories per rat per day.

	In food	In faeces	Per cent. calories in faeces
Heated food	37986	4064	10·7
Unheated food	39016	4250	10·9

There is thus shown to be practically no difference in the two cases, and it can be assumed that the seed does not carry any enzyme capable of breaking down the mucilage in the animal organism.

[1] *J. C. S.*, T. 1892, 527.

In order to confirm the above experiment, it was repeated with prepared mucilage added to the diet in addition to the linseed itself. In this way the percentage of mucilage in the diet was raised to 8 per cent., and any difference in utilisation in the two periods would probably be more marked than in the previous experiment, but no difference could be detected.

BACTERIAL ACTION.

There still remained the possibility that in the ruminants, where bacterial digestive action is great, the mucilage might be more completely broken down than in other animals. Unfortunately the preparation of mucilage on a sufficiently large scale to form any appreciable portion of a ration for a sheep or cow is impossible with only laboratory apparatus, but an attempt was made to obtain some information by laboratory experiments.

To a dilute solution of the mucilage, kept in a thermostat at 38° C., was added a small quantity of fresh caecum contents from a cow. The flask containing the mixture was arranged so that any gas evolved could be collected. In the first experiment from 2 gm. of mucilage in 250 c.c. water containing nutrient salts there were obtained about 70 c.c. of gas, of which 50 c.c. were absorbed by potash and the remainder was inflammable. The evolution of gas, however, quickly stopped, and when the contents of the flask were tested they were found to be strongly acid. The experiment was therefore repeated with the addition of a quantity of calcium carbonate to the solution In this case the gradual evolution of gas continued for a long period and in the course of a few hours 500 c.c. of gas were easily collected. About 90 per cent. of the gas was carbon dioxide, about 6 per cent. methane and the remainder oxygen and nitrogen. From the contents of the flask about 7 per cent. of the weight of mucilage taken could be distilled over as volatile acids when heated with dilute sulphuric acid, and in this distillate butyric and acetic acids were recognised. In the reaction flask there is also precipitated from the clear mucilage solution a certain amount of flocculent matter, and even after some time small quantities of mucilage could be precipitated by alcohol.

Attempts were made to use the mucilage for bacterial culture, like agar-agar on Petri dishes, to differentiate if possible between those bacteria which attack the mucilage and those which do not. The attempt was not successful, for it was found to be impossible to keep the mucilage at the right stage of hydration on the plate.

Summary of Action of Enzymes, etc.

Under laboratory conditions the mucilage is unattacked by the digestive enzymes, and even when fed to a non-ruminant animal 75 per cent. can be shown to pass through unchanged. As far as such a point can be demonstrated by laboratory experiments, it is however probable that the mucilage is attacked by intestinal bacteria, and, in ruminants especially, largely broken up in this way, with the evolution of gases and certain volatile acids among other products. This behaviour while unexpected at the beginning of the investigation is not inconsistent with its chemical character as a hydrated cellulose. A similar case is perhaps found in the substance agar-agar, which is readily attacked by bacteria, but is stated by Armstrong in his article on "Carbohydrates" in Allen's *Organic Analysis* to be practically indigestible by the human organism.

The above experimental results draw attention once more to the use of the term "soluble carbohydrates" in connection with feeding stuffs. In the usual routine analysis of foods many different compounds are grouped under this heading and are necessarily assigned one feeding value. Where the sugars and starches form the great bulk of the substances so grouped no great error results; but where, as in the case of linseed, the principal "soluble carbohydrate" is one of very different behaviour in the animal organism, the ordinary analysis may be misleading. Although it was impossible to prepare mucilage in sufficiently large quantities to carry out rigid feeding experiments on large animals, the results which were obtained point to a much lower actual feeding value for linseed mucilage than for starches and sugars, and this result is of considerable importance since popular opinion assigns to linseed such a high value, and even routine analysis necessarily gives it a value equal to the sugars and starches.

The best thanks of the author are due to Professor T. B. Wood for many valuable suggestions and for the interest he has taken in the work throughout.

PASTURE PROBLEMS: DROUGHT RESISTANCE.

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Owing to the exceptional drought of 1911, an excellent opportunity presented itself for studying the behaviour and power of resistance of the several species which contribute to the herbage of pastures and meadows. The very variable nature of the soils and pastures, which forms a striking and perplexing feature in regard to agricultural problems on the Cotswolds, contributes to make the area eminently suited for an investigation of this sort.

Quantitative analyses were made on a number of typical fields at different dates as the season advanced with a view to tracing the progressive relation between climatic conditions and the personnel of the several pastures. The results obtained from a small series of analyses have been published elsewhere[1]. It is now proposed to treat the question more fully in the light of a larger series of analyses which carries the investigation to the end of April 1912, the condition of the herbage in the spring being found to afford a valuable commentary on the effect of the drought.

Area Investigated: Soil.

The fields investigated were selected so as to exemplify the most frequent types of grass land met with on the Cotswold area. Most are on the farms of the R.A. College, Cirencester, of Mr Bruce Swanwick, Coates, at an elevation of about 430 feet above sea level. The soil in the case of these is derived from the Great Oolite, being a dry calcareous loam (10%—14% calcium carbonate) varying in depth from 6″ to 24″. The subsoil, except where otherwise stated, is brashy calcareous rock (15%—40% calcium carbonate). The results obtained from a few fields differently situated are given in some instances for the sake of

[1] Stapledon, R. G., "The effect of the Drought of 1911 on Cotswold Grass land." *Roy. Agric. College, Cirencester, Scientific Bulletin*, No. III. for 1911.

comparison. Really first-class grass land does not come under the scope of the present inquiry.

Type Fields: Particulars[1].

(*a*) Fields on R.A. College Farm.

A. No. 15.

Put down to grass about 1894 after sainfoin, with a mixture of the finer grasses and Dutch clover. It was renovated about six years ago with a sowing of perennial rye grass and Dutch clover. It is typical of much of the prepared sheep walks of the district and normally affords abundant keep. It was grazed heavily all through the summer with sheep and cattle.

B. No. 13 (*the Chapel Close*).

Put down to grass over fifty years, it now consists largely of endemic species, and is typical of meadow land on the thin soils. It maintains a high average yield of somewhat coarse hay. It was recently liberally dressed with farmyard manure and artificials.

C. No. 16.

Put down in 1895 with a mixture of meadow foxtail, cocksfoot, tall fescue, meadow fescue, perennial rye grass, rough-stalked meadow grass, alsike, red and white clovers. A high exposed field, periodically cut for hay. The soil, although shallow, contains a considerable amount of clay.

D. No. 12.

Under grass since 1862. It has been liberally dressed and mown constantly since 1888 with occasional two or four years rests. Typical of the prepared meadows on the deeper soils, maintaining an average of about 25 cwt. of hay to the acre.

E. No. 14.

Permanent pasture since 1892, carries sheep and cattle to which extra foodstuffs are fed.

[1] The capital letters serve as references to the Tables. Particulars as to soil-depth and calcium carbonate are shown in the Tables.

Portion (*a*). Owing to its position this part of the field receives considerable supplies of water by percolation from the College drainage system above. The herbage was consequently well maintained all through the summer, but was coarse.

Portion (*b*). Typical of the better classes of grazed pasture on the deeper soils.

(*b*) FIELDS SITUATE OTHERWHERE THAN ON THE R.A. COLLEGE FARM.

F. *Barren Rabbit Warren at Colesborne.*

This field is typical of much waste land in the Cotswolds occurring at elevations of 630 feet and upwards above sea level. It consists of a natural herbage which in the past has carried rabbits only. The soil is derived from the Inferior Oolite and ranges from 3″ to 6″, seldom attaining to 9″ in depth, and contains from 20% to 34% of calcium carbonate and is singularly bare of vegetation.

G. *Old Permanent Pasture at Latton, Cricklade.*

Rough herbage grazed by cattle; soil stiff alluvium resting on calcareous gravel.

H. *Old Permanent Grass at Dry Leaze, Cirencester.*

Gives hay of poor quality. Soil clay (about 1% $CaCO_3$), due to leaching out of calcium carbonate from Oolite soil cap.

I. *Irrigated Water Meadow, North Cerney, Cirencester.*

Very productive well-managed meadow. Since the conditions are the direct opposite to those obtaining on the thin soils under investigation, the field is peculiarly valuable for the sake of comparison.

K. *Four and Two Year Old Leys, Coates.*

Full particulars of the mixtures used are available and are given hereafter (p. 138).

BOTANICAL ANALYSES: METHODS.

(*a*) *Qualitative Analyses.*

A preliminary examination was made on each of the fields selected. The plan usually employed was that previously adopted on arable land[1].

Specific Frequency[2]. A number of readings are taken with a mesh $6'' \times 6''$. In the case of each reading the species present are noted without regard being paid to the individual frequency of the plants. The results are conveniently shown by the number of times each species occurs per 100 readings or by merely drawing up a list of the species in order of their relative frequency. This plan gives a good idea of the general character of a field and greatly facilitates the selection of typical plots. Since the analyses now under consideration were made for the special purpose of comparison at relatively frequent intervals typical plots of about 1/10th of an acre were chosen for each field and the samples for all the analyses taken from them.

(*b*) *Quantitative Analyses.*

Two methods have been employed:

(1) *Number of Plants to the Acre: Percentage Frequency.* These analyses were made by counting the individual plants in the manner prescribed by Armstrong[3].

(2) *Sorting and Weighing of Edible Herbage: Percentage Productiveness.* This method, although tedious, undoubtedly gives the best index of the nature of a field from an economic point of view. Two methods of sample-taking have been adopted: (*a*) Samples of grass (hay) are taken off the swards on the day of cutting, and by a process of mixing and discarding these are worked down to a convenient bulk, sorted and weighed; (*b*) numerous samples are taken by cutting the produce from within a mesh $6'' \times 6''$, and then sorting and weighing as before. This method has proved to be very satisfactory and expeditious, for by having a series of paper bags for the several species a large amount of preliminary sorting can be done upon the field during the process of sample-taking. The produce is kept upon moist blotters

[1] Stapledon, R. G., "Notes on the Weed Flora of some Arable land." *Roy. Agric. College, Cirencester, Scientific Bulletin*, No. II. for 1910.

[2] This method would seem to be generally applicable to Survey work where large areas are involved.

[3] Armstrong, S. F., "The Botanical and Chemical Composition of the Herbage of Pastures and Meadows." *Journ. of Agric. Science*, vol. II. Pt 3.

in the laboratory during final sorting, and is therefore weighed as green grass in a condition as nearly as possible similar to that in which it comes off the field.

Results given in terms of percentage frequency cannot, of course, be compared directly to those given by percentage productiveness. It will, however, be shown on some future occasion that the relationship obtaining between them throws considerable light on the relative absolute productiveness of the various species which constitute herbage when growing under different conditions.

RESULTS.

The results are best shown by considering those obtained by each method of analysis in turn. Discussion of their bearing on drought resistance is postponed, as far as possible, to the general conclusions at the end of the paper. Points of general interest are touched upon as they arise.

I. PERCENTAGE FREQUENCY.

(*a*) *Permanent Grass of over seventeen years standing.* (See Tables I and II.)

Field A. It is unfortunate that this field was not analysed by the above method in 1910 or during 1911 before the drought commenced. The specific frequency was however obtained in 1910. It is shown in the Table, and will be seen to be very different to that obtaining after the drought had taken full effect. The preponderance of Dutch clover was very marked during 1910, and the field then showed a closely compacted herbage; Armstrong's figures show that when Dutch clover is abundant the number of plants to the acre is very considerable, aggregating from 17 to 20 millions and upwards. As the April analyses give close on 10 millions of plants to the acre and the herbage was sparse and thin and nothing like up to par, it may be safely asserted that the clover prior to the drought would have approximated to 12 millions per acre. The dates of the analyses, viz. October 20th, 1911, February 20th and April 20th, 1912, show the condition of the field (1) right at the end of the drought before any appreciable recuperation had begun; (2) four months later when, under the ameliorating influence of late autumn and early winter rain very considerable improvement in the herbage was manifest; and (3) when the progress of recovery had continued for two more months. The

figures therefore depict the progress of recovery and not of destruction. The results, given in tabular form, are self-evident and do not call for comment.

TABLE I.

Progressive changes in the Botanical Composition of the Herbage on Field A.

Soil Depth 6″—8″ Calcium carbonate 12·7 %	Relative frequency	Thousands of plants per acre	Per-centage	Thousands of plants per acre	Per-centage	Thousands of plants per acre	Per-centage	Relativ frequen
Dates	July 1910	Oct. 20, 1911		Feb. 20, 1912		April 20, 1912		191
Lolium perenne	3	483	48·0	1587	23·1	1981	20·0	1
Poa pratensis	6					1742	17·6	2
Poa trivialis	4	2	trace	1218	17·7	696	7·0	5
Poa annua	—					479	4·8	6
Avena flavescens	2	9	·9	171	2·4	936	9·5	3
Cynosurus cristatus	1	72	7·1	502	7·3	384	3·7	7
Dactylis glomerata	5	59	5·8	196	2·8	195	2·0	8
Phleum pratense	trace	—	—	76	1·1	21	·2	trace
Agrostis stolonifera	—	21	2·1	—	—	—	—	trace
Agropyrum repens (*g*)	—	—	—	83	1·2	108	1·1	trace
Total Gramineæ		646	63·9	3833	55·6	6542	65·9	
Trifolium repens	1	72	6·9*	435	6·3*	765	8·2*	4
Medicago lupulina	traces	—	—		—	—	—	—
Trifolium minus		—	—	—	—	—	—	
Total Leguminosæ		72	6·9	435	6·3	765	8·2	
Ranunculus bulbosus (*g*)	2	89	8·8	1192	17·3	1720	17·4	2
Other weeds	7	195	19 4	1403	20·8	869	8·5	3
Total weeds		284	28·2	2595	38·1	2589	25·9	
Totals in millions of plants per acre	say **17** to **20**	**1·0**		**6·8**		**9·8**		

By February and April the following weeds were also present: *Cerastium triviale* (*g*), *Stellaria media*, *S. perennis* (*g*), *Cirsium arvense*, *Taraxacum officinale*, *Alchemilla arvenis* (*g*), *Geranium molle*, *Plantago lanceolata*, *Achillea Millefolium* (*g*), *Fumaria officinalis*, *Galium Aparine*, *Veronica arvensis*, *V. agrestis*, *V. Buxbaumii*, *V. hederaefolia*, *Capsella Bursa-pastoris*, *Euphorbia Helioscopia*.

* Corresponds to about 2·3 %, 2·1 % and 2·7 % of edible herbage respectively. Plants marked (*g*) gregarious and occur in considerable clumps together.

It should be pointed out that owing to the deplorable condition of the field, large bare patches (as much as 3′ to 4′ by 2′ or 3′) frequently occurring, sample-taking was rendered most difficult, therefore the

figures must be considered as showing tendencies only, and not as an accurate census of the field. Species marked (*g*) in the table had colonized the barren patches to a greater or less extent, notably by February and April. It was found impossible to differentiate accurately between the several poas till April 20th, when the final relationship between them is shown.

Several Fields. A number of fields were analysed by this method during April; the results obtained show their condition after recuperation has been carried a long way and afford a useful comparison to the end stage of field A. The chief features are briefly presented in the subjoined Table (p. 136).

In forming a proper estimate of the results it should be borne in mind that although the number of plants to the acre affords much information as to the effect of the drought, the fields cannot be indiscriminately compared with one another, on the strength of one analysis only, since every pasture type tends to have an optimum number of plants to the acre, which optima will vary very much for the several types. The physical condition of the pastures, when analysed in April, however, suggested that the aggregates given are far below the optima for fields F, A, and C, decidedly below it for E (*b*) and B, slightly below for D, and hardly at all for G and H.

It will be convenient here to mention the chief points brought out by the results:

(1) Other things being equal, the thinner the soil the greater the injury.

(2) Other things being equal, a crop of hay, and especially a good crop, would seem to have lessened even the final injury.

(3) Fields that have suffered most are seen to carry the largest percentage of weeds—more weeds occur on the grazed than on the hayed fields.

(4) Apart from the bearing of the figures on drought resistance it is seen that if the results are compared with those presented by Armstrong no very definite relation exists between the optimum number of plants to the acre and the merit of an old pasture. Probably however the optimum for the best old pastures would be found generally to fall between 19 to 23 millions of plants to the acre. Aggregates either much below or much above are only compatible with pastures of inferior type.

TABLE II. *Comparison of eight fields to show the relationship between soil-depth and treatment on the one hand, and number of plants to the acre and chief contributing species on the other. April* 20, 1912.

Fields	Soil depth	Extent of injury due to the drought	Hay or grazed	Thousands of plants per acre			Total in millions per acre	Dominant species Percentage frequency	
				Gramineae	Leguminosae	Weeds			
F	3″—5″	very great	barren rabbit warren	1·651 (69·4)*	29 (1·2)*	693 (29·4)*	**2·3**	*Agrostis vulgaris*.....	37·2
								Festuca ovina	10·5
								Poa pratensis	15·3
								Dactylis glomerata ...	4·4
								Cynosurus cristatus	·2
								Perennial weeds	19·7
								Annual weeds	9·7
A	6″—8″	very great	grazed	6·542 (65·9)	765 (8·2)	2·589 (25·9)	**9·8**	see Table I	—
E (*b*)	12″—16″	considerable	grazed	8·973 (61·7)	2·766 (19·2)	2·765 (19·1)	**14·5**	*Festuca ovina*	26·3
								Lolium perenne	20·7
								Trifolium repens	18·6
G	36″—40″ (alluvium on gravel)	hardly appreciable	grazed	12·675 (74·4)	2·047 (12·1)	2·265 (13·5)	**16·9**	*Festuca ovina*	21·8
								Agrostis stolonifera...	28·8
								Avena pubescens	6·8
								Trifolium repens	9·0
C	8″—10″ (sub-soil clay)	very great	Hay (5 cwt. to acre)	6·864 (59·0)	3·396 (28·9)	1·433 (12·1)	**11·6**	*Festuca ovina*	14·9
								Agrostis stolonifera...	12·8
								Festuca elatior	6·5
								Trifolium repens	28·8
B	8″—10″	slight in the hay, more considerable on aftermath	Hay (25 cwt. to acre)	13·307 (89·4)	740 (5·0)	849 (5·6)	**14·8**	*Bromus erectus*	29·3
								Festuca duriuscula ...	36·8
								Dactylis glomerata ...	3·2
D	12″—18″	ditto	Hay (20 cwt. to acre)	15·638 (82·4)	2·025 (10·7)	1·263 (6·9)	**18·9**	*Lolium perenne*	25·6
								Poa trivialis...	13·1
								Avena flavescens	13·0
								Trifolium repens	10·4
H	36″—38″ (clay)	hardly appreciable	Hay	19·233 (76·0)	4·464 (16·8)	1·964 (7·2)	**26·6**	*Festuca ovina*	24·6
								Poa trivialis	14·3
								Lolium perenne	10·6
								Trifolium repens	13·2

(*b*) *Leys:* 2—4 *years' duration.* (See Table III.)

The results given in Table III show both the mixtures used and the botanical composition of two leys which are compared to a natural tumble-down pasture of similar age—thus giving accurate information as to the relative success of the sown and endemic species.

The information derived from these figures is drawn upon in the general conclusions.

II. SPECIFIC FREQUENCY.

Barren Rabbit Warren at Colesborne.

The conditions that obtain here are eminently critical, every species that occurs on the area being *prima facie* a drought resister. Consequently it is of importance to learn not merely what species but what general growth forms have succeeded best. Analyses by the species method have been made over four characteristic areas. It is found that the chief contributing species amount to 76 in number. The percentage frequency is given for one area (see Field F, Table II), which shows: (1) the herbage to be excessively sparse, only giving 2·5 millions plants to the acre, and (2) the flora to be markedly non-gramineous, about 30% being a miscellaneous assemblage of dicotyledonous with a few monocotyledonous plants. With a view to gauging the success of the various growth forms the following classification based upon the species actually encountered on the fields analysed is of interest.

Review of the Morphological characters of the Flora.

1. Annuals	about 20 % of the total species
2. Biennials and Perennials:	
(*a*) With deep growing, generally thickened root systems. Vegetative organs tufted or erect	,, 25 ,, ,, ,,
(*b*) Deep rooted and with thickened creeping stems or rootstocks	,, 16 ,, ,, ,,
(*c*) Root depth variable: runners, stolons, rootstocks or offsets not much thickened or if so fleshy	,, 27 ,, ,, ,,
(*d*) Not very deep rooted: considerably thickened runners, stolons or rootstocks ...	,, 9 ,, ,, ,,
(*e*) "Bulbous" or tuberous plants ...	,, 2 ,, ,, ,,
(*f*) Shallow rooted non-creeping non-thickened hypogeal organs	,, 1 ,, ,, ,,

TABLE III.

Shows relation between Seed-Mixtures used and the Botanical Composition of the herbage of two Leys. The composition of a field that has tumbled-down naturally is given for comparison.

Situation	Leys at Cirencester				Tumble-down at Colesborne
	Sown 1910		Sown 1908		After Wheat 1908
Soil depth Cirencester 6"–9" Colesborne 4"—7"	Mixture in lbs. per acre	Percentage April, 1912	Mixture in lbs. per acre	Percentage April, 1912	Percentage July, 1911
Lolium perenne	12	38·6	5	31·1	—
Lolium italicum	10		3		—
Avena elatior	1	·5	1	·2	—
Avena flavescens	—	—	—	—	3·2
Dactylis glomerata	1	9·2	4	11·5	—
Phleum pratense	2	11·3	2	18·9	—
Poa pratensis	1	·9	—	2·1	20·0
Poa trivialis	—	—	—	—	—
Alopecurus pratensis	2	—	—	—	—
Festuca ovina (vars.)	4	3·2	2	3·1	68·8*
Festuca elatior	½	—	3	—	—
Festuca pratensis	2	—	4	·3	—
Cynosurus cristatus	—	—	—	·8	—
Bromus mollis (and *B. sterilis*)	–	·1	—	4·6	1·0
Agrostis stolonifera	—	—	—	·3	—
Agropyrum repens	—	—		·3	—
Total Gramineae		66·8		73·2	93·0
Trifolium repens	1½	9·5	2½	1·5	1·1
Trifolium pratense (and vars.)	8	4·0	3	·3	—
Trifolium hybridum	2	3·0	1	·2	—
Trifolium minus	½	1·1	—	-	—
Medicago sativa	1	·3	—	—	—
Medicago lupulina	1	—	—	—	—
Lathyrus pratensis	—	—	—	—	1·7
Total Leguminosae		17·9		2·0	2·8
Perennial weeds		8·2		10·4	4·2
Annual weeds		7·1		14·4	
Total weeds		15·3		24·8	4·2
Totals in millions of plants per acre		1·8		1·5	—

* = *Festuca duriuscula.*

By way of giving a general idea of the nature of the flora and emphasizing the above classification, examples of plants belonging to each class are given below. The figure against each species represents its specific frequency (i.e. the number of times it occurred per 100 readings—averaged from four areas).

Details of Flora classed as above showing Specific Frequency.

(g) Gregarious in groups together, (s) solitary.

1. *Linum catharticum* 29, *Alchemilla arvensis* 8, *Myosotis scorpioides* 3, *Arenaria serpyllifolia* 3, *Festuca sciuroides* 4, *Draba verna* 6, *Sonchus asper* 2, *Euphrasia officinalis* 5.

2. (*a*) *Dactylis glomerata* 4, *Taraxacum officinale* 14, *Poterium sanguisorba* 50, *Silene inflata* 3, *Plantago lanceolata* 3, *Geranium dissectum* 1.

(*b*) *Lotus corniculatus* 18, *Galium verum* 5, *Carex glauca* 47, *Brachypodium pinnatum* (g), *Achillea Millefolium* (g).

(*c*) *Carduus arvensis* 24, *Lathyrus pratensis* 8, *Agrostis vulgaris* 40, *Festuca ovina* 60, *Poa pratensis* 10, *Avena flavescens* 4, *Briza media* 6, *Prunella vulgaris* 16, *Ranunculus repens* 7, *Veronica Chamaedrys* 15.

(*d*) *Viola hirta* (vars. and hybrids 17), *Carduus acaule* 5, *Senecio Jacobaea* 30.

(*e*) *Ranunculus bulbosus* (s), *Orchis apifera* (s).

(*f*) *Anthoxanthum odoratum* (s).

The above classification can only be approximate, the distinctions being arbitrary; but it certainly shows:

(1) That drought resistance is not correlated with any one set of morphological characters.

(2) That the creeping habit associated with thickened underground vertical or horizontal organs is as efficacious, or even more so, on these thin soils than a penetrating root system.

(3) That certain annuals are very adaptable to conditions of drought: annual weeds constitute 9·7 % of the total herbage (Table II). They are mostly early flowering ephemerals[1].

It affords hints as to morphological types suitable for cultivating on barren land as a preliminary to establishing a remunerative herbage.

[1] Abundance of annuals is a noteworthy feature on most heath grass land. See Tansley, *Types of British Vegetation*, pp. 94—97. Camb. Univ. Press.

III. Percentage Productiveness.

(See Table IV.)

This comprises a series of analyses taken before the commencement of, during, and after the termination of the drought. The full results are set out in the Table (Table IV) herewith.

In order properly to substantiate the final conclusions something must here be said about the individual fields.

Field B. Bromus erectus is seen to have been more abundant in 1910 than in 1911. The reduction was due to heavy grazing and subsequently harrowing the aftermath in 1910. The effect of the drought has been to bring back the brome to its previous position despite endeavours to keep it in check. The frequency of *Festuca duriuscula* is greater than that of the brome, viz. 36·8 : 29·3 (see Table II), which shows how very productive the brome grass is on these soils. A comparison of the absolute productiveness of brome, cocksfoot and rye grass was made (by weighing the produce of 100 plants of each) with the result:

Erect brome : cocksfoot : perennial rye grass :: 140 : 100 : 57.

Field C. Shows that even on thin soils, if somewhat retentive of moisture, Dutch clover is not completely killed; here, although giving only 3°/₀ of the hay, it had advanced to about 12°/₀ of the edible herbage by the Spring. (See Table II, showing 28°/₀ frequency = about 12°/₀ productiveness.)

Field E. (*a*) is the only field which shows an increase of clover from June to October. This must be due to the fact that a supply of moisture is fortuitously maintained here which kept up a good supply of coarse gramineous herbage, with the result that both shade and moisture were provided for the clover.

Field I. The flora is similar to that of other water meadows, the chief feature being excess of Yorkshire fog, rye grass and the creeping buttercup, accompanied by a paucity of clovers[1]. In the present connection it is instructive to note the good yields provided by soft brome, tall oat, crested dog's-tail and meadow barley grass; all grasses met with

[1] E.g. at Chester. See also Dr Fream, *Journal of Linnean Soc.* (Bot.), vol. xxiv. 1888. The association of Yorkshire fog and creeping buttercup with pastures on deep retentive soils is a common occurrence and detracts from the merit of acres of grass land in the West of England.

Fields	B					C	D		E (a)		I
Hay or grazed	Hay (25 cwt. to acre)					Hay (5 cwt. to acre)	Hay (20 cwt. to acre)		Grazed (coarse herbage)		Hay (2 ton to acre)
Water content	Dry					Dry	Slightly moist		Decidedly moist		Irrigated water-meadow
Soil depth	6″—8″—10″					8″—12″	12″—18″		12″—18″—22″		—
Dates	June 17 1910	May 20 1911	June 3rd 1911	Oct. 31 1911	April 20 1912	June 6 1911	June 10 1911	April 1 1912	June 9 1911	Oct. 31 1911	June 10 1911
Lolium perenne	2·2	1·4	6·9	6·0	4·0	19·7	35·0	40·0	17·0	21·3	30·2
Dactylis glomerata	8·5	6·7	10·2	21·5	9·9	21·9	22·4	13·0	24·3	32·3	8·0
Alopecurus pratensis	—	—	—	—	—	—	2·9	10·0	—	—	3·2
Avena flavescens	1·3	1·0	1·8	1·0	·3	1·8	12·6	+	·2	·5	2·0
Avena elatior	—	—	—	—	—	—	—	—	—	—	5·3
Avena pubescens	·2	·3	·1	—	·4	·9	—	—	·5	—	—
Poa trivialis	1·7	4·4	2·8	3·5	1·0	1·3	5·1	+	2·8	1·3	6·3
Poa pratensis					·9	3·0		+	—	—	—
Festuca duriuscula	8·1	13·8	14·8	17·0	14·9	1·6	1·8	22·0	6·1	8·4	2·2
Festuca elatior	—	—	—	—	—	21·1	—	—	—	—	—
Holcus lanatus	·6	·8	·4	—	·1	·6	1·6	+	29·3	15·0	22·0
Cynosurus cristatus	·4	—	·5	·5	·2	1·4	1·8	+	1·9	1·3	3·5
Anthoxanthum odoratum	—	—	·9	—	—	—	—	—	·4	—	·6
Bromus mollis	·3	6·7	1·8	—	1·6	—	2·6	+	—	—	4·6
Bromus erectus	62·0	44·8	45·3	41·0	58·1	6·6	—	—	—	—	—
Hordeum pratense	—	—	—	—	—	—	—	—	—	—	4·5
Agrostis stolonifera	—	·1	—	1·0	·2	—	—	+	·4	4·5	2·0
Aira caespitosa	—	—	—	—	1·7	—	—	—	—	—	—
Other grasses	—	—	—	—	—	—	—	—	—	—	2·7
Total Gramineae	85·3	80·0	85·5	91·5	93·3	79·9	85·8	85·0	82·9	84·6	97·1
Trifolium repens	9·2	7·7	5·7	2·0	1·5	3·0	6·3	10·0	6·3	8·5	·4
Trifolium pratense		1·0	·6	·2	—	—	5·2		2·8	1·8	
Lathyrus pratensis		1·4	·3	·8	·7	·3	—		—	—	—
Total Leguminosae	9·2	10·1	6·6	3·0	2·2	3·3	11·5	10·0	9·1	10·3	·4
Ranunculus bulbosus	5·5	8·8	7·9	3·0	4·2	12·8	2·0	5·0	8·0	5·1	—
Ranunculus repens		—	—	—	—	—	—		—	—	2·5
Other weeds		1·1	—	2·5	·3	4·0	·7		—	—	—
Total weeds	5·5	9·9	7·9	5·5	4·5	16·8	2·7	5·0	8·0	5·1	2·5

+ the species thus marked under field D have together contributed 22 % to the herbage.

in moderate quantity on dry situations. Although not having succeeded very well (except *B. mollis*) under the exceptional conditions of the summer of 1911, they are usually good drought-resisters. This shows that ability to resist drought is not incompatible with a capacity to succeed under conditions the direct antithesis to those obtaining on dry thin soils. Again, despite the fact that Yorkshire fog, creeping buttercup and marsh thistle attain to great luxuriance under humid conditions, it is remarkable that not inconsiderable quantities of all of them (though stunted specimens) occur on the barren pastures at Colesborne. It is evident therefore that drought resistance is not of necessity an absolute character, depending on morphological adaptation, but in many cases is but an outcome of the plant's inherent vitality.

General Conclusions.

The foregoing analyses, in conjunction with a number of close observations made on other fields in the immediate neighbourhood, justify the following conclusions:

(*a*) *General.*

(1) The critical soil depth seems to have been about 9″, pastures on soils of less depth having suffered to a far greater extent than those on soils of 10″ and upwards. Soils of 14″ and 18″ have produced, in many cases, very good yields of hay of good quality.

(2) Pastures on soils of about the critical depth have suffered more when closely grazed than when a crop of hay has been taken. In the case of one field analysed (A, see Table I) the reduction in the herbage was remarkable, for by October 22nd the aggregate number of plants on the field was but little over one million, having fallen from something between 16 and 20 millions as a direct result of the drought. Other analyses have shown a very different relationship between the component species on a field in October to what obtained before the drought began.

(3) The fields that suffered the greatest damage were those which in 1910 carried large quantities of Dutch clover. This plant died out in a wholesale manner, large bare patches being left on which the dead runners of the clover could be seen. On these patches the finer grasses, such as rough-stalked meadow grass, crested dog's-tail and golden oat grass had almost failed. (Fields A and C are examples.)

(4) As the season advanced the bare patches began to be colonized

by seedlings of the bulbous buttercup (*Ranunculus bulbosus*), daisy, and mouse-eared chickweed (*Cerastium vulgatum*), and some by runners of the persistent yarrow. On some fields, especially long leys, large amounts of bindweed (*Convolvulus arvensis*) and dandelion (15% on one field) were in evidence. Arable land weeds also began to put in an appearance.

(5) The extent of recuperation by the middle of February was surprising. On Field A (see Table I) the aggregate flora had increased since the middle of October from about one million to over six millions plants to the acre. By the middle of April the recovery had been carried a considerable stage further, the total number of plants to the acre then being 9·8 millions. The manner of recuperation was very different for the different fields, and would seem to have depended chiefly on the potential vitality of the stoloniferous plants (e.g. *Trifolium repens* and *Poa pratensis*).

(6) Recovery on the bare patches was, however, not satisfactory, the clovers and valuable grasses having done practically nothing to reclaim them. Instead they were colonized by little closed associations of daisy, buttercup, or mouse-eared chickweed seedlings. Some arable land weeds had also established themselves in force, chief of these being the little parsley piert (*Alchemilla arvensis*), a weed normally only found in small quantity, if at all, in pastures of this district, but one very common on sainfoin leys and arable fields generally: here it had colonized areas of yards together. Speedwells, chickweed, fumitory, sun spurge, cleavers, and the like, were also much in evidence. Couch (*Agropyrum repens*) had established itself on many fields, generally taking a part in the colonization of the bare patches. The same is true of *Poa annua*. Moss, which was in evidence during the summer, had increased extensively by the spring on grazed fields where it occupied positions not taken up by Angiospermic species on the hard bare patches.

(7) Most of the grasses (notably *Lolium perenne*) ripened and seeded early. Much ripe seed was carried with the hay and so could not benefit the parent field; but when the hay was fed to stock on grass land the seed was shed, as was rendered apparent in the autumn by the presence of brilliant green patches of rapidly growing seedlings where the pens had previously stood.

(8) The severe frost during the early part of February does not seem to have retarded the rapid recovery of the pastures.

(*b*) *The several Species.*

Clovers. In general they fell to a very small bulk during the summer—on grazed fields on thin soils only giving about 2% of the edible herbage (footnote Table I). Under hay their productiveness was directly proportionate to the yield. On all the fields, except one, they attained to their maximum relative productiveness towards the middle of May. Before the hay was cut the productiveness decreased[1], and showed a progressive decline in the aftermath till October. On Field B the clover fell by 7% relative to other plants between May 20th and October 31st.

Dutch Clover (*Trifolium repens*) did not begin to recuperate till after the middle of February. On the thinnest soils, especially where no hay was taken, the plant was killed to such an extent that recovery to date has been very slight and it may never regain its former position. On fields of better soil-depth (10″ and over) or even on thin soils more retentive of moisture (e.g. Field C, Tables II and IV) it has evinced great powers of recuperation, in one instance having come on fourfold since the summer. The following facts point to the conclusion that the continuous scorch of the sun had as deleterious an effect on the growth of this plant, especially with regard to its productiveness through the summer, as had the actual drought, thus:

(1) its almost total failure on some closely-grazed fields;

(2) its much greater failure in the hay crop on meadows which produced a very poor, than those which produced a very good, yield of hay; and

(3) its progressive falling off in bulk on the aftermath, the only field where it maintained an average yield till October being one where not only was a good supply of water available, but the herbage was coarse and shade-giving. (See Table IV E (*a*).)

Red Clover (*Trifolium pratense*). The deep-rooted varieties have proved moderately resistant on two and four-year-old leys. The greater failure of even the deep-rooted varieties than of Italian rye grass on short leys is probably to be attributed to the sun: the more so as the clovers have failed more in the aftermath than in the hay. Many leys are now to be seen consisting almost entirely of rye grass. The greater failure of the clovers in the leys may, however, be in part due to the

[1] On Field B the standing crop was estimated on May 20th as equal to 25 cwt. of hay, it did not increase at all by June 3rd when it was cut. The coarser grasses came on but the clovers had begun to decline.

baneful effect, claimed by many authorities, of rye grass upon clover, an effect more likely to be apparent during a critical than a normal season.

Alsike (*Trifolium hybridum*). On leys of two and four years duration this contributed appreciably to the bulk of the herbage and would thus seem to be a useful plant for leys even on these thin soils.

Yellow Suckling Clover (*Trifolium minus*) and Black Medick (*Medicago lupulina*). Both of these plants were less frequent than usual on fields that normally carry them. On leys the medick sown in 1910 was not present at all in samples taken in April 1912.

Bird's-foot Trefoil (*Lotus corniculatus*) and Meadow Vetchling (*Lathyrus pratensis*) showed themselves very resistant. On barren pastures at high elevations and on shallow-soiled fields they came up in great quantity in the spring of 1912. *Lathyrus* would seem to have bulked more heavily in the 1910 hay crop than in that of 1911—but it was more abundant in the 1911 aftermath than usual. Speaking generally, it is not a good aftermath plant.

Grasses.

Perennial Rye Grass has everywhere shown itself wonderfully resistant and persistent. All the analyses show a progressive relative increase from May to October, when it reached its maximum relative productiveness, thence falling in position as less resistant species began to recover and the quantity of the creeping resisters increased. Compared with its productiveness under ideal circumstances it would seem to have lost in absolute productiveness (on these soils this season) as 70 : 47[1]. The ubiquitous distribution of this grass is noteworthy. It was present on nearly all the pastures investigated in quantities from 6% to 30%. It does not however seem to have established itself to any extent on the barren uplands at Colesborne, but even there on fields that have been put down to grass it ranks about fifth in order of frequency[2].

On many fields it showed a tendency to emit short stolons, bearing little tufted plantlets at the nodes. The inflorescence frequently approximated to the type *racemosum*[3].

[1] Ascertained by weighing 100 plants of each, setting large against large and small against small and so forth till the full number is obtained. The ratio is given on the false assumption that cocksfoot is here fully productive, so that the rye grass figure should be less in proportion to the depressed productiveness of cocksfoot.

[2] Nor has it established itself much on tumble-down pastures. It may perhaps not be endemic on the Cotswolds.

[3] I am indebted to Mr S. F. Armstrong for identification of typical specimens collected in 1910.

Cocksfoot (*Dactylis glomerata*). Like other resistant species it came on progressively from May to October, then falling off through the spring. It now occupies practically the same position as it did before the drought. This shows that the individual plants bulked well through the drought: but by virtue of its caespitose habit it has not been able to win ground at the expense of the less resistant species.

Hard Fescue (*Festuca duriuscula*) on fields where it may be regarded as endemic has proved very resistant and surprisingly luxuriant, having in one instance contributed as much as 14% to the hay. On such situations it has behaved in a manner similar to cocksfoot, except that in virtue of a somewhat creeping habit it has been able to occupy more ground. Consequently, although falling slightly since October, it now stands considerably higher than it did in 1910 (as 14·9%: 8·1%).

On leys that have come under observation where this and other varieties of sheep fescue have been sown, they have not proved successful, nor have they got hold of the ground even in proportion to the amount of seed sown (Table III).

On tumble-down leys of equal age and on fields apparently similar the endemic variety has, however, established itself naturally in great quantity (see Table III).

From this it would seem:

(1) That varieties exotic to the neighbourhood are of no value here.

(2) That the endemic species is either very capricious in establishing itself on one field and not on another, or that sowing down with a full mixture serves to completely prevent it coming in naturally on a field.

In 17 years on Field C (sown with a full mixture) it only contributed 1·6% to the herbage as opposed to 68% in four years on the tumble-down.

(3) It is probable that if large amounts of seed from the endemic variety could be collected, results as satisfactory as those recently obtained by the use of wild Dutch clover might be forthcoming.

Erect Brome Grass (*Bromus erectus*) has given a great bulk of herbage all through the drought, and since it has a decidedly creeping habit it has increased its hold on fields since 1910. This plant is greatly reduced, and may ultimately be almost entirely eliminated by constant and close grazing. On one field where constant grazing had reduced it to an unobservable amount it may now be seen in little tufts scattered

all about the field, thus affording singular testimony as to its capabilities as a drought resister.

Where hay is required on the thinner soils the presence of this grass is a very important factor. When abundantly present good yields of hay (dominated by this grass) have been harvested. Compare, for instance, 25 cwt. from Field B with 45% erect brome, to 5 cwt. from Field C with 6·6% of that grass. The soils of both fields are below the critical point. Erect brome also, by the shade it offered to the clovers, contributed to their better yield on the former field. It was noted also that the soils cracked less when protected by the brome, and carried a smaller amount of miscellaneous herbage. It must be pointed out, however, that this dominance of a relatively coarse bulky grass tends actually to depress the absolute productiveness of most of the other component species, not even excepting cocksfoot. Like hard fescue it is endemic on the Cotswolds; it does not, however, seem to come in so rapidly on tumble-downs as the fescue, but when fully established is equally dominant and far more productive. Two distinct varieties have been observed, the normal with rather narrow involute leaves, and one with much broader leaves and longer marginal hairs placed further apart. It is everywhere attacked to a variable degree by *Ustilago hypodytes*, the diseased plants never flowering and assuming a characteristic stunted very distichous form. In 1912 the attack was as bad as ever. As some difference was seen on the different manurial plots, which have been in continuance for over 23 years on Field B, analyses were made, some of which are here given:

Kainite alone	13% smutted
Kainite and superphosphate	10% „
Unmanured	10% „
Kainite, superphosphate, and sodium nitrate	9% „
Sodium nitrate	no smut.

Soft Brome (*Bromus mollis*) was very abundant on many hay fields, but seeded and died down very early, so that it did not itself bulk largely in the hay crop (except in exceptionally wet fields or on irrigated meadows, when the abundance of moisture lengthened its period of vegetative growth), but it baulked the growth of better and later grasses, and was, therefore, in many cases a factor in reduced hay yield. As a result of early and prolific seeding in 1911 it is now to be seen coming up in great quantity even on fields where previously it was present in small quantities only.

Tall Fescue (*Festuca elatior*), on the thin soil of Field C, withstood the drought well, giving 21% of the meagre hay crop harvested there. On the leys (Table III) it has not shown to advantage, but this was apparently due to a failure on the part of the seeds to establish themselves in the first instance.

Meadow Fescue (*Festuca pratensis*) is not suited to these thin dry soils and has nowhere shown to advantage. It has completely died out in 17 years on Field C, and only contributes a trace to the herbage on short leys.

Timothy (*Phleum pratense*) on leys of two and four years duration has proved itself surprisingly resistant. It gave a percentage frequency only second to rye grass (Table III), nor was the produce very poor or the plants strikingly "bulbous." The absolute productiveness per unit plant[1] compared to that of cocksfoot was as 57·9 : 100—the figure for the grass growing under ideal conditions being 75 : 100. Consequently timothy would appear to be of value as a short ley grass even on moderately thin soils (8″ and upwards).

Meadow Foxtail (*Alopecurus pratensis*) on the thin soils does not establish itself, and is of no use as an ingredient for even short leys. On deeper soils (12″ and upwards) it contributes well to the herbage of older fields. On Field D it is abundant, but was reduced to 2·9% of the hay during the summer, but the drought there did it no lasting harm, as by the spring it was very luxuriant and gave 10% of the ground herbage.

Tall Oat (*Arrhenatherum avenaceum*), although having the reputation of a drought-resister, does not contribute largely to the herbage of this district, and when used on short leys does not establish itself satisfactorily. As an endemic grass it occurs in quantity at the bottoms of dry hedges, and in young plantations it colonizes large patches under the slight shade of larch and other trees even when the conditions are very dry (soil 5″—9″). This suggests that the grass is a dry-place shade-grass, and its failure on poor pastures is due more to the lack of shade than to simply arid conditions.

Yorkshire Fog (*Holcus lanatus*), with regard to productiveness, has suffered considerably. Large amounts occurred only where moisture was maintained by fortuitous circumstances (Fields E (*a*) and I, Table IV), but even when so placed it decreased in quantity as the season advanced. As small stunted specimens it withstood the drought even on the barren pastures at Colesborne.

[1] See footnote under Rye Grass.

Crested Dog's-tail (*Cynosurus cristatus*) undoubtedly suffered very much, and has not maintained its reputation as a drought-resister. It has bulked best on the wetter, deeper-soiled fields. On Field A it dropped from a dominant position in 1910 to fifth position in 1912. It only contributed ·2% of the herbage on barren pastures at Colesborne and ·4% on the deeper-soiled closely grazed E (*b*). Careful search was made for the grass on another grazed field where it was formerly very abundant, but the number of plantlets found was surprisingly few. The above facts suggest that the excess of sun has played an important part in its reduction.

Golden Oat Grass (*Avena flavescens*) fell very low on the grazed fields below the critical soil depth, but has seldom been completely destroyed, and has recovered remarkably since the autumn. It maintained an average yield on some of the deeper-soiled meadows. It is indigenous to the neighbourhood and occurs to some extent on the barren pastures at Colesborne, and has come in naturally, both on the four-year tumble-down and on Field C.

Fiorin (*Agrostis stolonifera* and *A. vulgaris*) on many of the shallower-soiled fields certainly increased considerably above the normal as the result of the drought. *A. vulgaris* is seen to be the most resistant grass on the barren pastures at Colesborne, but it is very unproductive there.

The Poas have behaved differently on different fields, but dropped much in productiveness through the summer.

Smooth-stalked Meadow Grass (*Poa pratensis*). A very widely distributed endemic grass has come in naturally in good amount on Field C (Table IV), and on four-year tumble-down (20%, Table III). On short leys its abundance does not show any relation to the amount of seed sown, consequently it is doubtful if it is ever worth including in mixtures. It gave a very sparse herbage through the summer, but it was not killed and was the first grass to regain position early in the autumn. On Field A (Table I) it has changed from sixth in order of frequency in 1910 to second in 1912, contributing 17·6% of the plants to the acre, although on October 31st it was discernible only in traces—thus it has won much ground at the expense of less resistant species.

Rough-stalked Meadow Grass (*Poa trivialis*) was reduced to a minimum on the thin-soiled fields and in part killed right out; even on the deepest it was reduced far below par, but on these has done much to regain position during the spring.

Miscellaneous Plants.

Bulbous Buttercup (*Ranunculus bulbosus*) has shown great failure in the production of 1912 corms; this has, however, been more than counterbalanced by the numerous seedlings produced.

Yarrow (*Achillea Millefolium*) on the thin soils produced very little herbage through the summer. The stolons were not killed and the plants gained much ground during the spring.

Summary.

The following brief summary may be given on the aetiology of drought resistance as exemplified by the conditions obtaining in 1911.

From an agricultural point of view the value of a plant as a drought-resister is seen to be measurable by two standards: (1) Its power to give a good yield all through the period of drought; (2) its ability through living through the summer without materially adding bulk to the herbage to recuperate when the conditions become less severe. It has been shown that the phenomenon of drought-resistance is not associated with any one set of morphological characters, but that various growth forms are met with amongst the most successful plants. Further, a number of plants have shown themselves very tolerant, although having no apparent modifications to assist them, in which case there can be no doubt that their power of resistance is a simple outcome of their inherent vitality. Consequently it is perhaps dangerous to assign too great an importance to the possession of apparently useful modifications. A fair correlation is however seen to exist between a plant's *manner* of resistance and its growth form, as the following classification shows.

Perennials.

(1) Plants which give a high absolute productiveness all through the summer:

(*a*) Spot bound (caespitose and erect) plants which do not gain actual ground on the fields they occupy, i.e. their percentage frequency is not appreciably higher in the spring following the drought than it was in the spring preceding it, e.g. *Dactylis glomerata* (deep root system), *Lolium perenne* (inherent vitality), *Phleum pratense* (two- and four-year leys), *Poterium sanguisorba* and *Onobrychis sativa* (deep root system).

(*b*) Slightly creeping plants which gain ground on the fields they occupy, e.g. *Bromus erectus* (deep rooted and thickened), *Festuca duriuscula* (thickened), *Festuca elatior* (deep rooted), *Lathyrus pratensis* (thickened offsets).

(*c*) Creeping plants which gain much ground on the fields they occupy, e.g. *Brachypodium pinnatum*, *Carex glauca* and *Lotus corniculatus* (deep rooted and thickened creeping root systems and stolons).

(2) Plants which give a poor absolute productiveness through the summer, but which, in the main, are not killed:

(*a*) Spot-bound plants which become productive with the advent of better conditions, but cannot win new ground, e.g. *Trifolium pratense* (vars.) and *T. hybridum* (short leys)

(*b*) Those which evince great recuperative powers, rapidly regaining and often winning position on the ground, e.g. *Poa pratensis* (moderately deep but not much thickened stolons), *Agrostis stolonifera* (shallow stolons), *Avena flavescens* (creeping slightly thickened root system), *Achillea Millefolium* (moderately deep, thickened, creeping stock) and plants of the type *Potentilla anserina* and *Prunella vulgaris* (shallow runners and inherent vitality).

(*c*) Those which remain small and stunted, peopling adverse habitats to some extent, but never productive, e.g. *Holcus lanatus*, *Anthoxanthum odoratum* and *Cynosurus cristatus* (sometimes due to inherent vitality).

(3) Plants which are not drought resisters, being killed under trying conditions, and which show on practically all situations a depressed productiveness through the summer and recover in the spring in inverse proportion to the severity of the habitat:

(*a*) When not destroyed regain position in the spring, e.g. *Trifolium repens* (shallow runners) and *Poa trivialis* (shallow decumbent runners), *Alopecurus pratensis* (slightly stoloniferous, *vide* patches of this grass on deeper-soiled pockets on shallow fields). *Arrhenatherum avenaceum* (creeping root system: gregarious).

(*b*) Even when not killed unable to regain ground in the spring, e.g. *Festuca pratensis*, *Cynosurus cristatus* (despite deep root system), *Phleum pratense* (except on short leys and pastures where stunted specimens are endemic), *Holcus lanatus* (except on pastures where stunted specimens are endemic).

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THE EFFECT OF PARTIAL STERILISATION OF SOIL ON THE PRODUCTION OF PLANT FOOD.

PART II. THE LIMITATION OF BACTERIAL NUMBERS IN NORMAL SOILS AND ITS CONSEQUENCES.

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IN our earlier communication[1] we showed that bacteria can no longer be regarded as the only active inhabitants of the soil. We obtained evidence of another group of organisms, detrimental to bacteria, and differing from them by their larger size, slower rate of multiplication under soil conditions, and lower power of resistance to heat and to antiseptics. These are more readily killed than bacteria, and we regarded their suppression as an important factor in determining the increased bacterial activity known to set in after soil has been partially sterilised. Such properties as we were able to ascertain agreed with those of the protozoa, for which we were thus led to look: we found representatives of each of the three groups, ciliates, flagellates and amoebae. We therefore supposed that some of these protozoa constituted the detrimental organisms indicated by our experiments.

Subsequent experiments made by ourselves, by Goodey, Martin, and others, have shown that numerous kinds of protozoa occur in the soil, but they have also revealed a new difficulty, that of ascertaining precisely which kinds are leading their trophic life in the soil and which kinds are present only as cysts. As this problem is not likely to be solved till much more work has been done from the zoological side we decided in the meantime to continue our experiments from our own point of view and to determine the effect on soil fertility of these

[1] This *Journal*, 1909, **3**, 111–144.

conflicting groups of organisms. These experiments form the subject of the present paper.

The investigation falls naturally into two parts. In the first instance it is necessary to determine the effect on bacterial numbers of the presence of the detrimental organisms. It follows as a simple deduction from the existence of these organisms that the number of bacteria present per gram of soil at any given time does not depend primarily on the temperature, the water supply or other conditions of the soil, but on the difference in activity of the two groups. Thus a rise of temperature favours not only the bacteria but also the detrimental organisms, and if the latter happen to be favoured more than the former the bacterial numbers will fall. Experiment shows that this deduction is correct. No sort of relationship can be traced between bacterial numbers and temperature, the numbers sometimes rising, sometimes falling, and sometimes being unaffected by rise of temperature (Table II). Increases in the amount of soil moisture may or may not increase the numbers of soil bacteria. These erratic effects are not peculiar to our own soil, but are general and have caused much perplexity and not a little controversy among soil bacteriologists in the past (§ 5). They are entirely explicable on our view that bacterial numbers simply represent the difference in activity of bacteria and the detrimental organisms. Further confirmation is found in the fact that in partially sterilised soils (from which the detrimental organisms are absent) the bacterial numbers increase in a regular manner with rise of temperature and of water content (Figs. 1, 2). Increases in the amount of organic matter in the soil also fail to increase bacterial numbers to a corresponding extent and may indeed, lead to soil "sickness" as shown in previous papers[1]. But when the detrimental organisms are suppressed by partial sterilisation the expected rise in bacterial numbers sets in and the phenomena of "sickness" are not seen.

Similarly any other factor favourable to the growth of living organisms may effect a reduction in bacterial numbers through bringing about a development of the detrimental organisms. *Vice versa*, causes which in themselves are unfavourable to growth may nevertheless lead to increases in bacterial numbers through suppressing the detrimental organisms.

In the second place we have attempted to trace the connection between bacterial numbers and soil productiveness. Other circumstances

[1] This *Journal*, 1912, **5**, 27, 86.

being equal, the greater the numbers of bacteria the more rapid is the production of ammonia and of nitrate in the soil. But we find that an accumulation of either of these substances, and especially of ammonia, tends to stop further accumulation, even though the bacterial numbers increase, so that the relationship between ammonia production and bacterial numbers is no longer seen. No sharp relationship can be looked for in any case because our methods of counting bacteria afford only very rough approximations. There is evidence, however, that the fluctuations shown by the gelatine plate methods correctly reflect the fluctuations of the total numbers of decomposition bacteria; when used for this purpose the data are distinctly valuable. It may happen, however, that the productiveness of the soil is limited by some other factor such as temperature, water supply, insufficiency of calcium carbonate, of phosphates, potassium compounds, etc. and that the additional supply of nitrogenous food is therefore ineffective to raise the crop. Increases in bacterial numbers only increase the productiveness of the soil when the conditions are such that the increased numbers can make more ammonia and nitrate, and when no other limiting factor intervenes to prevent this extra nitrogenous plant food from causing more plant growth.

We have also dealt with some of the objections raised against our main conclusions that organisms exist in the soil detrimental to bacteria, and that the bacterial numbers at a given moment are determined by the mutual interaction of these conflicting groups. It has been asserted that no such organisms exist, and in particular that ciliates and amoebae could not lead a trophic life in the soil. Others have supposed that our results are due to an improvement in the bacterial flora brought about by partial sterilisation, either through the suppression of certain forms detrimental to food-making bacteria or through a stimulus resulting from the treatment and transmitted to the descendants of the surviving organisms. No experimental proof is offered in support of these views and we have been able to adduce direct evidence against them. Our results have also been attributed to the presence of bacteriotoxins in the soil, it being assumed that these toxins are decomposed by the antiseptics (chiefly toluene vapour) that we used. We have also considered the possibility of changes in the colloids and other materials that may be supposed to coat the particles of the soil.

Our experiments show that the detrimental factor has all the attributes of living organisms. Thus it is a positive factor (*i.e.* it is not a lack of some essential or desirable condition); it is capable of

growth and of extinction; once extinguished it does not arise again until some of the untreated soil is added. The difficulty of defining life is well known, but we think the sum of the properties points conclusively to a living organism. Further, every deduction we have made from the existence of the two conflicting sets of organisms has been justified by experiment, while each new experimental fact that has come to light is found to fit in readily. Our identification of the detrimental organisms with certain soil protozoa is only provisional and may be modified by subsequent zoological surveys of the soil fauna; for the present, however, we adhere to it because it accords with all the known facts.

Definite evidence could be obtained against the view that the bacterial flora is improved by partial sterilisation. The flora as a whole is certainly more effective in bringing about various decompositions, but this arises from an increase in numbers and not from an increased efficiency of the organisms. As a matter of fact the organisms lose in efficiency, and, when the old flora is put under the same conditions as the new by inoculating it into partially sterilised soil, then it attains numbers much higher than the new and brings about more decomposition (§ 24, Table X).

We have failed to find bacteriotoxins in our soils which, it should be noted, are fairly rich in calcium carbonate. Further, the deductions made from the bacteriotoxin hypothesis do not all come out right: *e.g.* the toxins ought to accumulate in partially sterilised soils where there is great bacterial activity, but they do not (§ 22). Certain of the observed facts can be explained on the hypothesis, but as new facts are brought out it becomes necessary to attribute new and more remarkable properties to the toxins in order to account for them.

There is more difficulty in dealing with the changes that might be induced in the soil colloids because it seems possible to attribute to colloids practically all the properties of living organisms. The evidence, however, seems to be against this view as a complete explanation of all the phenomena.

Of course we do not assert that bacteriotoxins do not exist in any soils or that the condition of the soil colloids plays no part in determining the bacterial population, or that partial sterilisation has no other effect on the soil except to destroy the harmful organisms. On the contrary we have shown that heating the soil to 100° C. or higher temperatures brings about considerable decomposition and considerably

alters the soil as a medium for the growth of micro-organisms[1]. Even the milder treatment with antiseptics does not leave the soil wholly unchanged but produces effects some of which are dealt with in a later paper. Some of our results could be explained on the supposition that heat, toluene, etc. set free some substance essential or favourable to bacteria, the lack of which in the untreated soil was limiting their numbers. But many of our results cannot be explained in this way. The only hypothesis covering all the facts is that in normal soils bacteria are not the only active organisms, but that larger organisms occur detrimental to them, and the bacterial population of the soil at any moment is determined by the mutual interaction of these conflicting groups.

EXPERIMENTAL PART.

I. The effect of the detrimental organisms on the bacterial numbers in the soil.

§ 1. During the course of our investigations we have frequently had occasion to bring in soils from the field, submit portions to partial sterilisation processes, keep them in bottles under constant conditions of moisture and aeration at the laboratory temperature and make periodical counts of the bacteria by gelatine plate cultures. Some of the results are collected in Table I. The numbers in the untreated soils often vary in rather an erratic manner, rising and falling for no obvious reason; they show much more regularity in the partially sterilised soils, however, and generally rise steadily to a maximum at which they either remain or begin to fall; only rarely do they fluctuate as in the untreated soils.

As the partially sterilised soils had been kept alongside of the untreated soils and were equally moistened and aerated, the difference in behaviour cannot be attributed to any external cause but must be put down to some condition present in the untreated and absent from the partially sterilised soils.

[1] It seems impossible to convince some soil biologists that the organic matter of the soil suffers decomposition on heating to high temperatures, thereby changing the soil as a medium for the growth of organisms. Again and again we find distinguished investigators steaming soil under pressure and assuming that it has undergone no change. Conclusions drawn from experiments with these steamed soils are applied to ordinary unheated soils, not only without modification, but apparently without seeing the need for any modification. And yet for the past thirty years chemists have been giving proofs of this decomposition.

§ 2. A simple explanation is afforded by the consideration that bacteria are not the only active inhabitants of the soil but are accompanied by larger organisms detrimental to them and keeping them in check. On this view the number of bacteria in the soil at a given moment represents the balance of activity of the two sets of organisms and is therefore not connected in any simple way with the temperature, water supply, etc. These factors can increase the bacterial numbers only if they shift the balance in favour of the bacteria, and whether or not this can happen in a particular case is only discoverable on our present knowledge by actual trial.

TABLE I. *Numbers of bacteria in untreated and in partially sterilised soils.*

Millions per gram of dry soil.

	At start	End of 1st period	End of 2nd period	End of 3rd period	End of 4th period
		16 days	30 days	74 days	
Soil 1.—Untreated soil	27	10	10	45	
Soil treated with CS_2* ...	2	17	53	121	
		15 days	110 days	170 days	200 days
Soil 2.—Untreated soil	13	9	4	9	12
Soil heated to 65° C. ...	13	21	37	45	60
		40 days	100 days	160 days	500 days
Soil 3.—Untreated soil	11	16	9	13	6
Soil treated with toluene	2	43	41	43	18

* In all cases it must be understood that 0·5—1 % of antiseptic is added to the soil (except where otherwise stated) and left to act for about 30 hours, and then allowed to volatilise completely. Sterilised water is then added to bring the soil to the correct degree of moistness. Throughout this paper except in Table III the bacterial numbers are stated in millions per gram of dry soil.

Small changes in conditions may therefore raise or lower the numbers to a disproportionately large extent, or, on the other hand, they may be without action. Thus in the untreated soil we expect erratic results. But in the partially sterilised soils we expect and obtain more regular results. The detrimental organisms are now killed and the surviving bacteria are free to multiply and to show the normal behaviour towards changes of temperature, etc.

The influence of soil temperature on bacterial numbers.

§ 3. In order to test this deduction a series of experiments was started to ascertain the effect of temperature on the numbers of bacteria

in the soil. A quantity of fresh arable soil was put through a 3 mm. sieve and divided as uniformly as possible into a number of portions which were kept in bottles plugged with sterile cotton wool. Half of the samples then received 1 per cent. of toluene which, after 24 hours, was allowed to evaporate by spreading out the soil on sterilised paper in a closed room for 30—40 hours. No smell could then be detected. The soil was returned to the bottles and received sufficient sterilised water to bring all the samples up to a uniform moisture content representing 60—70 per cent. of the saturation value. Some of the bottles were stored in a shed where the temperature was low but variable (5°—12° C., unfortunately we had no thermostat working at 10° C.) while others were stored in incubators maintained respectively at 20°, 30°, 40° and 50° C. Samples were periodically taken out for analysis, the bacteriological results of which are given in Table II. Some of the results are plotted in Figs. 1 and 7.

§ 4. In the untreated rich soil (No. 2) kept at 20° the bacterial numbers rose steadily for 20 days and then fell off; at 30° the numbers fell during the whole time, while at 50° the fall was rapid and complete. In the untreated poor soil (No. 1), kept at 20°, the numbers fell off from the outset and are consistently below those in the same soil kept at a lower temperature. At 30° almost the same results were obtained except on one occasion, at 40° there is a partial drop which becomes complete at 50°. In the richest soil the same general result is obtained, the numbers fell off at 20° and are always below those in the soil kept at 5°—12°. There is some outburst of activity after the soil has been stored for three weeks at 30°, but this does not persist; at 40° there is a marked falling off. The most remarkable feature, however, is that, with one exception, the numbers are no higher at 20° or 30° than at the lower temperature, but on the contrary they are generally lower.

Thus it appears that, in these untreated soils, rise of temperature does not exert the favourable influence on bacterial numbers that might have been expected; any beneficial effect is only temporary. In other words the detrimental organisms become more active than the bacteria as the temperature rises to 20°.

A wholly different set of results, however, is obtained where the soils have been previously exposed to the vapours of toluene. In the soil *RC* the numbers steadily rise at 20° and are always much higher than at the lower temperature; the numbers also rise for a time at 30°, but do not get so high as at 20°. In the richest soil very similar

results are obtained; there is a steady rise at the low temperature and a much quicker rise at 20° (the temporary drop after 25 days we are unable to explain). At 30° the rise is even more rapid but is not maintained, while at 40° the conditions become less favourable still. The poor soil behaves like the others, except that even at 20° the high numbers cannot be maintained, but fall to 30 millions per gram. All

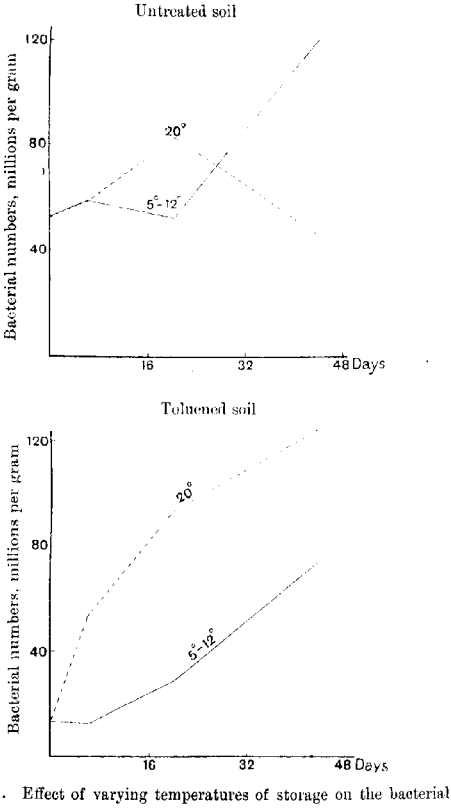


Fig. 1. Effect of varying temperatures of storage on the bacterial numbers in the soil. Soil *RC* (Table II).

these partially sterilised soils stand out in sharp contrast with the untreated soils in that there is a much more rapid increase in bacterial numbers when the temperature is raised to 20° than when it is kept

TABLE II. *Effect of temperature on the numbers of bacteria in untreated and in sterilised soils.*

Millions of bacteria per gram of dry soil

1. A poor soil containing 14 % water, 0·18 % N, 3·16 % $CaCO_3$ and losing 4·6 % on ignition.

Temperature, ° C.	Untreated soil				Soil treated with toluene			
	At start	After 6 days	After 28 days	After 58 days	At start	After 6 days	After 28 days	After 58 days
5°–12°	11	11	9	7	3	8	27	28
20°	11	8	4·5	6	3	50	30	30
30°	11	8	9	6	3	43	24	31
40°	11	9	2	7·5	3	12	4	6
50°	11	4	0·7	1	3	2	1	1

2. A richer soil, *RC*, containing 16 % water, 0·37 % N, 0·57 % $CaCO_3$ and losing 11·05 % on ignition.

Temperature, ° C.	Untreated soil				Soil treated with toluene			
	At start	After 6 days	After 20 days	After 44 days	At start	After 6 days	After 20 days	After 44 days
5°–12°	53	58	52	121	13	11	30	76
20°	53	58	82	46	13	54	92	125
30°	53	19	25	24	13	60	88	87
40°	53	7	9	9	13	43	49	7
50°	53	1	13	1	13	2	10	1·5

3. A very rich soil, *OxL*, containing 40 % water, 0·63 % N, 1·9 % $CaCO_3$ and losing 17 % on ignition.

Temperature, ° C.	Untreated soil				Soil treated with toluene			
	At start	After 13 days	After 25 days	After 70 days	At start	After 13 days	After 25 days	After 70 days
5°–12°	65	63	41	32	8·5	73	101	137
20°	65	41	22	23	8·5	187	128	182
30°	65	27	50	16	8·5	197	145	51
40°	65	14	9	33	8·5	148	52	100

The amounts of nitrogen present as ammonia and nitrate on the various dates are given in Table XVI.

The percentages of nitrogen, calcium carbonate and loss on ignition, are calculated on the air dried soil in each instance.

at 5°—12°; the bacteria being free to multiply now that the detrimental organisms are killed. It will further be noticed that the organisms counted by the method adopted cannot long survive a temperature of 50°, suffer considerably at 40°, and do not flourish as well at 30° as at 20°.

§ 5. The ineffectiveness of increased temperatures to increase bacterial numbers in ordinary untreated soils is not a peculiarity of our soils. It is seen also in the experiments of Hiltner and Störmer, of Engberding and of Conn, and has been observed also by Löhnis. The bacterial counts made by Hiltner and Störmer[1] at intervals during a year on field plots show no tendency for bacteria to increase as the temperature rises, the August results being no higher than those obtained in February. Some of their figures are:

Bacteria in millions per gram (Hiltner and Störmer).

	Cropped land, grass and clover	Fallow land, cultivated	
		No dung	Dung*
May 10th, 1901 ...	8·3	8·0	11·0
Aug. 27th ,, ...	3·2	4·2	10·5
Oct. 18th ,, ...	6·4	4·0	11·0
Feb. 1st, 1902 ...	6·6	4·1	9·3
June 12th ,, ...	8·1	5·7	7·2
Aug. 18th ,, ...	4·9	4·1	8·4

* The dung was applied in July at the rate of 130 to 140 Centner pro Morgen (10 to 11 tons per acre).

Engberding[2] made a similar but more extensive series of counts of the bacteria in plots of ground at intervals during the year and has published his results in very complete form, giving the mean temperature of the soil during the week when each sample was taken, the moisture content, details of rainfall, etc. Here again no connection whatsoever can be traced between the bacterial numbers and the temperature, in fact it often happens on dates when the moisture contents are similar that the bacterial numbers are higher when the temperature is lower, as in the following examples:

[1] L. Hiltner and Störmer, Studien über die Bacterienflora des Ackerbodens, mit besonderer Berücksichtigung ihres Verhaltens nach einer Behandlung mit Schwefelkohlenstoff und nach Brache, *Arb. Biolog. Abt. Land- u. Forstwirtschaft. Kais. Gesund.* 1903, Bd. **3**, Heft 5.

[2] Diedrich Engberding, Vergleichende Untersuchungen über die Bakterienzahl im Ackerboden in ihnen Abhängigkeit von äusseren Einflüssen, *Centr. Bakt. Par.* II, 1909, **23**, 569—642.

	Soil temperature (mean for week) ° C.	Per cent. of moisture at time of sampling	Millions of bacteria per gram of dry soil
Plot 1 *a*. Uncultivated and uncropped—			
Aug. 14th, 1907	16°·6	16·58	13·09
Sept. 26th „ „	14°·1	16·55	20·85
Plot 8 *b*. Fallow—			
April 29th, 1908	9°·9	18·69	26·12
May 7th „	13°·4	17·31	14·13
June 2nd „	18°·9	17·87	11·77

Conn[1] found that the numbers of bacteria in his plots were high in February, fell in summer and rose again in autumn, and was able to draw up a very neat curve showing the changes of bacterial numbers with the season. He realises that this result implies two conflicting sets of organisms, and suggests tentatively that "there may be two groups of bacteria in the soil, one flourishing in winter, the other in summer. In this case the conflict between these two groups may explain the occurrence of two seasons, one in early fall, the other in winter, when bacteria are particularly numerous." This hypothesis would account for Conn's results but not ours; on the other hand, our hypothesis accounts not only for our own results but for Conn's as well.

Löhnis[2] has shown that certain bacterial changes, such as the decomposition of cyanamide and urea, take place more rapidly in the soil in spring than in summer. Thus in May 1907 with a temperature of 9°, and a moisture content of 11·2 per cent., decomposition proceeded more rapidly than in the following August when the temperature was 15°·8 and the moisture content 15·8 per cent.

The influence of moisture content in bacterial numbers.

§ 6. Samples of soil were put up in baskets of silver wire suspended in covered beakers containing water. One sample was fairly dry, a second was moist and contained a very satisfactory amount of water for bacterial development, while the third sample just dipped into the water and was therefore wet without being waterlogged. A parallel set of three put up in the same way had previously been treated with toluene and then received sufficient water to make the percentages

[1] H. J. Conn, Bacteria in Frozen soil, *Centr. Bakt. Par.* II, 1910, **28**, 422—434.

[2] Löhnis, F. and Sabaschnikoff, A., Ueber die Zersetzung von Kalkstickstoff und Stickstoffkalk, *Centr. Bakt. Par.* II, 1908, **20**, 322—332. Other cases are quoted in Löhnis, *Handbuch der Landw. Bakterologie*, 1910, p. 596.

equal to those in the untreated set; the water added contained an extract of the untreated soil carrying bacteria in order to make the bacterial flora as nearly as possible comparable in the two cases. The soils were all kept in the incubator at 25° C. Counts of the bacteria were made periodically by the gelatine plate method; the results are set out in Table III, and plotted in Fig. 2.

TABLE III. *Numbers of bacteria in soils containing varying amounts of water.*

Millions per gram of soil as taken from baskets, and not dried.

(*a*) Barnfield dunged plot.

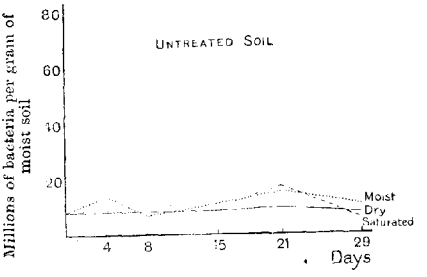
	After 3 days	After 5 days	After 10 days	After 19 days	After 27 days
Untreated soil, dry	4	4	13	6	10
,, moist ...	14	24	37	77	20
,, saturated	26	38	38	52	40
Toluened soil, dry	3	6	10	17	9
,, moist ...	38	26	48	71	33
,, saturated	29	45	77	58	33

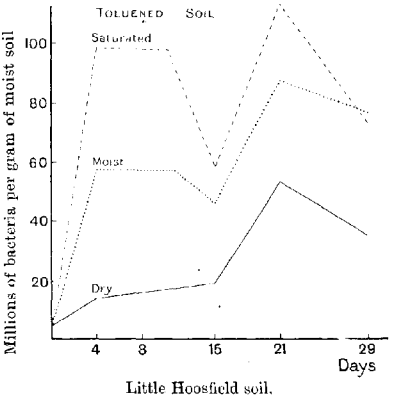
(*b*) Little Hoosfield.

	At start	After 4 days	After 8 days	After 15 days	After 21 days	After 29 days	Moisture present
Untreated soil, dry	8	8	7	8	9	8	8·8
,, moist ...	—	14	7	10	15	10	13·9
,, saturated	—	8	10	9	16	5	—
Toluened soil, dry	5	15	—	20	55	37	7·4
,, moist ...	—	58	47	—	90	80	12·0
,, saturated	—	101	98	60	116	75	—

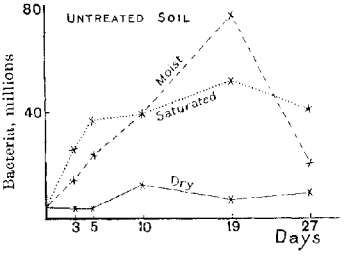
Reference to the curves shows that in the untreated Barnfield soil (the richer of the two) there has been but little multiplication when the moisture content is low, a more rapid rate when more water is present and, for a time, a still more rapid rate when the soil is wet. But this higher rate of multiplication is only maintained for a short time. After the fifth day the increase in numbers is small and the curve bends over, showing unmistakeably the operation of a limiting factor. In the toluened soil there is no evidence of this limiting factor. The curve

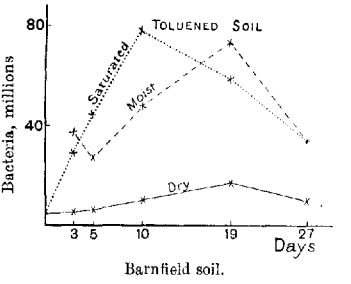
for the wet soil resembles that for the moist soil but shows a greater rate of bacterial multiplication, as one would expect. When the maximum point is reached the falling off in numbers is slower than in the untreated soil.





Little Hoosfield soil.





Barnfield soil.

Fig. 2. Effect of variations in moisture content on the bacterial numbers in untreated and partially sterilised soils (Table III).

The addition of moisture is without effect on bacterial multiplication in the untreated Hoosfield soil where a limiting factor is plainly at work, but it leads to regular increases in bacterial numbers in the toluened soil. Apart from two low readings on the fifteenth day, the numbers fall on to a fairly regular curve.

II. SOME PROPERTIES OF THE DETRIMENTAL FACTOR.

§ 7. A series of experiments was now undertaken to discover some of the properties of the detrimental organisms with a view to facilitate identification. We decided, however, to work on a more general plan than was strictly necessary for this purpose, and to proceed as if we knew only the existence of a factor detrimental to bacteria but knew nothing as to its nature. We were thus enabled at once to ascertain some of its properties, to obtain still further evidence of its biological nature, and to answer some of the objections that have been raised to our previous work.

Reverting to §§ 3 and 4 and to Table II dealing with the effect of soil temperature on bacterial numbers: these results show that the factor is something positive and definite occurring in the untreated soil and not a negative factor such as lack of nutrient or other condition essential or desirable for growth. For it is difficult to see how a negative factor could cause a *drop* in the rate of multiplication at 20°; it would be more likely to set a limit so that the rate would be the same as at the lower temperature.

The moisture results of § 6, Fig. 2, Barnfield soil, also show the existence in the untreated soil of a positive hindrance to bacterial

growth. The falling off in numbers in the moist soil once the maximum is reached may be attributed to lack of food. But *the failure to reach the maximum* in the wet untreated soil can only be attributed to the presence of an active detrimental factor; for in the equally wet toluened soil the maximum is speedily attained.

The effect of the toluene is therefore not due to any foodstuff or stimulant, etc. that it may liberate, but to the extinction of something that was actually hindering bacterial development. (See also § 28.)

The experiments fall into three groups dealing respectively with:

1. The determination of the processes by which the detrimental factor can be put out of action, its extinction being indicated by the subsequent rise in bacterial numbers.
2. The biological nature of the factor.
3. Its non-bacterial nature.

Methods of extinction.

§ 8. (*a*) *Temperature.* The detrimental factor has a fairly sharp extinction point between 55° and 60° but it is also put out of action at lower temperatures if the heating is prolonged. Table IV gives the results of bacterial counts made in soils that had been heated for specified times at given temperatures and then stored under favourable conditions of moisture, aeration, etc. It will be observed that a temperature of 56° is sufficient in the first soil, 55° is barely sufficient in the second but 65° is ample, whilst in the third case a temperature of 50° maintained for one hour temporarily threw out the factor and the same temperature maintained for ten hours apparently extinguished it.

§ 9. On the other hand we failed to find any definite extinction point at low temperatures; there is, however, considerable difficulty in cooling soil owing to its low conductivity. When the cooling was made really effective by pouring liquid air on to soil contained in a Thermos flask and leaving it there to evaporate ((*a*) in Table V) the detrimental factor was put out of action and after 14 days the bacterial numbers were 60 millions per gram in place of 29 in the untreated soil. But the suppression was only temporary, and after a time the factor reasserts itself so that at the end of 42 days the numbers of bacteria were down even below the level in the untreated soil. On the other hand, when the soil was put into narrow test tubes and immersed in liquid air for half an hour the detrimental factor did not suffer and the bacterial numbers remained the same as in the untreated soil ((*b*) and

(*c*) in Table V). Immersion of test tubes of soil in solid carbon dioxide for about 2 hours was sufficient in one case to suppress the detrimental factor temporarily but not in another. More prolonged immersion in ice and salt was also successful in one case but the temperature of melting ice produced no change.

TABLE IV. *Effect of heat on the detrimental factor.*

Temperature of heating	Time of heating	Millions of bacteria per gram of soil: At start	After 7 days	After 21 days	After 68 days	After 142 days	
Unheated	—	11	10	12	11	4	
40°	—	7·5	9	10	7·5	3	Factor not extinguished
56°	—	2	14	16	37	45	Factor extinguished
		At start	After 15 days	After 120 days	After 180 days	After 210 days	
45°	—	13	9	4	9	12	
52°	—	15	11	9	13	23	Factor not extinguished
55°	—	5	5	3	13	73	?
65°	—	13	21	37	15	60	Factor extinguished
		At start	After 13 days	After 53 days	After 105 days	After 245 days	
Unheated	—	—	8	9	13	12	
50°	1 hour		26	26	15	16	Factor suppressed but apparently not extinguished
50°	12 hours	—	15	16	36	20	Factor temporarily extinguished

(The amounts of nitrogen present as ammonia and nitrate on the various dates are given in Table XV.)

§ 10. Our conclusion is that when the soil is cooled the detrimental factor is temporarily put out of action, and the extent to which it suffers depends on the effectiveness of the cooling. A long exposure to a moderately low temperature may be more effective than a short exposure to a much lower temperature. The factor is not permanently extinguished but reappears after a time.

§ 11. (*b*) *Rapid drying.* Soil was exposed in a thin layer in a hot room at 35°—38° for varying intervals and then moistened and stored in

bottles in the usual way. The results of the bacterial counts are given in Table VI; they show that 10 days' drying throws the factor out of action, but only temporarily, for the bacterial numbers fell again before 120 days had elapsed, indicating that the factor had become active once more. Another lot of soil exposed to 10 days' hot bright sunshine during June, 1911, behaved in a similar manner. There is a distinct resemblance between these effects, and those observed when soil is heated to 50° for an hour or cooled to a low temperature. In all of these cases the treatment falls somewhat short of what is wanted for complete extinction of the detrimental factor, and the result is a temporary suppression of the factor, followed by a re-establishment which appears to be complete.

TABLE V. *Effect of low temperatures on the detrimental factor.*

Cooling agent	Millions of bacteria per gram of dry soil					Ammonia* and nitrate produced	
	Approximate temperature	Length of exposure	At start	After 14 days	After 42 days	After 14 days	After 42 days
Liquid air (*a*)...	−180° C.	½–1 hour	8	60	12	—	—
,, (*b*)...	—	,,	—	26	27	—	28
,, (*c*)...	—	,,	8·2	26	28	—	25
Solid CO_2 (*a*) ..	−60° C.	2 hours	7	43	18	23	24
,, (*b*)...	,,	,,	—	26	13	22	26
Ice and salt (*a*)	−18° C.	6 hours	7	10	33	20	24
,, (*b*)	—	,,	—	37	57	22	24
Ice (*a*).....	0° C.	8 hours	9	21	19	21	26
,, (*b*).....	,,	,,	—	24	23	20	23
Untreated (*a*) ..	—	—	11	31	21	20	26
,, (*b*)...	—	—	—	27	25	22	26

In all cases the ammonia was by distillation with magnesia *in vacuo* as described in this *Journal*, 1910, **3**, 233; and the nitrate by the zinc copper couple method (this *Journal*, 1912, **5**, 32). The results are invariably stated as parts per million of dry soil.

* N as NH_3 varies from 2 to 3 parts, showing that nitrifying organisms were unaffected.

§ 12. This result is easy to explain on the view that the factor is biological: the treatment kills many of the detrimental organisms but not all, and the survivors subsequently multiply to their original density; in the meantime, however, the bacteria have a tolerably clear field for development. The result is difficult to explain on any toxin hypothesis unless one can assume that a toxin which is incompletely decomposed has the power of reproducing itself after a time; other hypotheses appear to present similar difficulties (*e.g.* see § 30, p. 184).

TABLE VI. *Effect of rapid drying on the detrimental factor.*

Method of drying	Millions of bacteria per gram of soil					
	At start	After 30 days	After 70 days	After 120 days	After 210 days	
Arable soil						
Untreated	11	5	(18)	11	...	
24 hrs at 35°–38°	5	7	18	11	7	Factor not extinguished
5 days ,,	4	9	17	9	9	,, ,, ,,
10 days ,,	—	12	25	13	10	Factor temporarily suppressed
10 days sunshine	2	18	22	7	11	,, ,, ,,
	At start	After 26 days	After 42 days			
Richer soil, *RC.*						
Untreated	27	28	39			
10 days at 35°–38°	7	37	59			

	N as NH_3, parts per million					N as NH_3 and nitrate*, parts per million				
	At start	After 30 days	After 70 days	After 120 days	After 210 days	At start	After 30 days	After 70 days	After 120 days	After 210 days
Arable soil										
Untreated	—	—	1	1	—	19	34	24	39	—
24 hrs at 35°–38°	7	1	1	1·5	2	25	31	37	44	56
5 days ,,	9	1	2	1·5	2	27	37	48	48	55
10 days ,,	7	2	2	2·5	1·5	25	39	57	61	81
10 days sunshine	3	0·5	1·5	0·5	2·5	21	31	42	44	58
	At start	After 26 days	After 42 days			At start	After 26 days	After 42 days		
Richer soil, *RC.*										
Untreated	5	2	3			91	105	105		
10 days at 35°–38°	15	3	2			96	139	141		

* In order to save space the nitrate figures are not given separately, but are added to the NH_3 figures to yield these totals. The amount of nitrate in any particular case can be readily seen by deducting the corresponding ammonia figure from the total.

§13. (*c*) *Antiseptics. Organic Liquids.* The action of organic antiseptics on the soil is not entirely simple. A small amount of ammonia is immediately produced by some process we have not yet investigated. Subsequently, after the antiseptic is completely removed from the soil,

there sets in a rapid production of ammonia which remains as such because the nitrifying organisms are killed. The bacteria growing on gelatine plates are greatly reduced in numbers by the action of the antiseptics, but they increase to a very considerable extent afterwards.

§ 14. Organic liquids that do not possess marked antiseptic properties also cause an immediate liberation of ammonia. This may be (but is not invariably) followed by an increased rate of production of ammonia which is converted into nitrate showing that the nitrifying organisms have survived. There may also be an increase in bacterial numbers. Neither the increased production of ammonia nor the increased rate of multiplication of bacteria (when these phenomena set in) is nearly so marked as in the case of strong antiseptics, and it is not clear whether this is a mild instance of the usual case, or a different type of action altogether. The simplest view is that only the liquid form of these substances is capable of killing the detrimental factor, the vapours being much less potent. As the liquid comes into contact merely with a small part of the soil the partial sterilisation effect is produced only to a restricted extent.

§ 15. Table VII shows the effect of hydrocarbons on the soil, approximately one per cent. by weight being used in each case. This was left to act for two days on the soil in tightly closed bottles and was then allowed to evaporate by spreading the soil in a thin layer for some 48 hours. The soil was then moistened and put up in bottles in the usual way. The benzene ring compounds show marked antiseptic properties, killing the nitrifying organisms and the detrimental organisms, and allowing the bacteria subsequently to multiply considerably (the xylenes did not completely volatilise from the soil and appear somewhat to have checked the subsequent bacterial development). Four parts per million of ammonia are produced immediately, and there is a subsequent development of 26 parts in 26 days.

The open chain compounds, however, belong to the second type (§ 14). They cause practically the same initial production of ammonia—5 parts per million—and there is also a subsequent production of ammonia, which, however, amounts to 12 parts instead of 26 in the 26 days. None of the compounds kills the nitrifying organisms. The subsequent effect on the bacterial numbers is variable: pentane causes no increase[1], hexane a marked one, and heptane a smaller one.

Cyclohexane is intermediate in action between the open chain and the benzene ring compounds.

[1] The only case of this action we have observed. See footnote, p. 215.

TABLE VII. *The effect of various organic liquids on bacterial activity in the soil.*

100 grams of soil received approximately 1 gram of liquid. Soil from Lucerne ley contained 12 % water, 0·13 % N, 1·8 % $CaCO_3$, and lost 4·9 % on ignition.

Hydrocarbons	Bacteria after 26 days, millions per gram of dry soil	Effect on nitrification	Parts per million of dry soil		
			NH_3 immediately produced	NH_3 in soil after 26 days	NH_3 and nitrate produced in the 26 days
Untreated	10·4	—	—	2	4
Open chain—					
Pentane	11·3	unaffected	5	1	12
Hexane	33·6	,,	5	2	13
Heptane	17·1	,,	5	1	12
Ring—					
Cyclohexane	lost	much inhibited	6	15	17
Benzene ring—					
Benzene	57·3	entirely suppressed	4	33	26
Toluene	60·0	,,	4	34	26
o-Xylene	37·1	,,	4	29	23
m-Xylene	30·4	,,	4	27	19
p-Xylene	35·3	,,	4	23	12
	After 27 days		NH_3 and nitrate produced in 27 days		
Untreated	10·5	—	4		
Methyl alcohol	20·7	unaffected	0		
Ethyl alcohol	29·5	,,	6		
Tertiary butyl alcohol	66	much inhibited	34		
Amyl alcohol	105	entirely suppressed	17		
Other substances—					
Untreated	10·4	—	4		
Acetone	14·3	unaffected	0		
Ether	30	somewhat checked	19		
Petrol	32·4	unaffected	10		
Alicyclic derivatives					
Untreated	14·2	—	3		
Bases—					
Pyridene*	250	entirely suppressed	154		
Collidene*	4	,,	430		
Lutidene*	6·6	,,	196		
Other substances—					
Thiophen	65	entirely suppressed	32		
Toluene	68	,,	31		
Nitrobenzene*	5	,,	7		
Benzaldehyde*	37	,,	0		

* These substances could not be removed from the soil by volatilisation.

(The rest of the Table is on p. 173.)

§ 16. The alcohols also fall into two groups, methyl and ethyl alcohols being relatively ineffective, while tertiary butyl and amyl alcohols are of the same order of effectiveness as toluene. Further investigation is necessary to decide whether the action is precisely the same as that of toluene, because of the possibility that the traces of alcohol left in the soil may serve as food for the bacteria and so bring about an increase in numbers; it is difficult otherwise to account for the 105 millions of bacteria in the soil treated with amyl alcohol.

Acetone is apparently inert; ether is distinctly active but not nearly so potent as toluene; petrol is less active. In another experiment on another soil both ether and chloroform were practically as effective as toluene.

All these substances, like the hydrocarbons, cause a small immediate liberation of ammonia from the soil.

§ 17. Pyridene behaves in a remarkable manner, causing an enormous rise in bacterial numbers and in the amount of ammonia present in the soil. It was impossible to remove all the pyridene by simple evaporation and a certain amount remained in the soil; the very high amounts of ammonia and bacteria indicate that some of this has been decomposed. This result has been obtained on several occasions and always with the purest pyridene obtainable from Kahlbaum; it is the more striking in that pyridene is very stable to ordinary reagents, almost entirely resisting the attack of nitric acid, sulphuric acid, the halogens, etc. Collidene and lutidene also give rise to large amounts of ammonia in the soil. The manurial value of pyridene has been demonstrated in an earlier experiment[1].

Thiophen behaves exactly like toluene. This result is obtained only when the soil is in so fine a condition that the toluene vapour can penetrate freely. In pot experiments where the soil is lumpy thiophen may prove more effective[2].

Nitrobenzene and benzaldehyde could not be removed from the soil.

§ 18. *Inorganic antiseptics.* There is considerable difficulty in securing uniform distribution of inorganic antiseptics in the soil because of their non-volatile nature; we have therefore confined ourselves to those that give off poisonous gases. Bleaching powder, calcium sulphide, and hydrogen sulphide in moderate quantities all behave like toluene as shown in Table VII, while sulphur dioxide (in moderately strong dose), bromine, and flowers of sulphur, all proved too drastic under the

[1] E. J. Russell and F. R. Petherbridge, this *Journal*, 1912, **5**, 106.

[2] *Loc. cit.* p. 110.

conditions of the experiment; whether in smaller quantities they would behave like calcium sulphide we did not determine.

TABLE VII (*cont.*). *The effect of various inorganic antiseptics on bacterial activity in the soil.*

Arable soil containing 15 % water, 0·18 % N, 3·16 % $CaCO_3$ and losing 4·6 % on ignition. 100 grams of soil received approximately 0·25 gram of antiseptic.

	Bacteria after 30 days, millions per gram of dry soil	Effect on nitrification	Parts per million of soil	
			NH_3 immediately produced	NH_3 and nitrate produced in the 30 days
Untreated	8·6	—	—	12
Calcium sulphide...	42·5	entirely suppressed	1	18
Hydrogen sulphide	64·5	?	9	16
Bromine	4·3	?	8	3
Flowers of sulphur	4·3	suppressed	0	0
Untreated..... ...	11·3	—	—	23
Sodium sulphite ...	10·3	unaffected	1	18
Bleaching powder	32	entirely suppressed	8	34
Sulphur dioxide ...	0	,,	0	5

The addition of one per cent. of quicklime was also found to partially sterilise the soil, producing the same kind of effect as toluene and other agents. It caused at first a depression in bacterial numbers, and also in the nitrifying organisms, but later on, when it was converted into carbonate, the usual increase took place both in numbers and in the amount of decomposition. This forms the subject of a later communication.

§ 19. All the experiments in which the antiseptic was removed from the soil lead to the same conclusion. Whenever the substance used is sufficiently potent to kill the nitrifying organisms it also puts the detrimental factor out of action, so that after it is removed from the soil, the numbers of bacteria and the rate of production of ammonia both increase to a marked extent. To this rule there is no exception.

To the converse statement there are exceptions: distinct increases in bacterial numbers and rates of ammonia production are sometimes obtained even when the nitrifying organisms are not all killed. The increases are not as marked as before, and the cases can all be explained on the view that the vapours of these substances are much less poisonous to micro-organisms than the liquid states.

We may conclude that those substances only which are capable of acting as antiseptics in the soil possess the power of suppressing the harmful factor.

§ 20. *The mode of action of the toluene.* The extinction of the harmful factor by toluene is complete even when only small quantities of toluene are used. There was no consistent difference in the bacterial population of portions of a poor soil that had been treated with 0·25, 0·5, 1, 2 and 4 per cent. respectively of toluene (Table VIII). 0·25 per cent. proved wholly insufficient to "wet" the soil, 4 per cent. on the other hand "wetted" it completely. It thus appears that the action is between the vapour and the soil, not the liquid and the soil.

In the richer soil 0·25 per cent. of toluene proves equally effective with the others for 30 days but not for 74 days. It was clear, however, that the vapour had not penetrated the whole of the soil as some of the nitrifying organisms escaped and set up a brisk nitrification after some 20 or 30 days had elapsed. Even 1 per cent. did not wholly exterminate these organisms but it so depressed them that they did not produce any measurable amount of nitrate till after the 30th day.

0·25 per cent. of carbon disulphide also proved insufficient to penetrate the soil, as seen by the fact that it only reduces the initial bacterial numbers to 9 instead of 2 millions per gram. It is therefore less effective than 1 per cent., but on the other hand, 1 per cent., which does not thoroughly "wet" the soil, is *more* effective than 4·4 per cent. which does. The conclusion to be drawn is that action is complete when the vapour has reached all parts of the soil, this point being indicated by the immediate depression of the bacterial numbers to the minimum number represented by the spores, and the suppression of the nitrifying organisms. The extinction of the harmful factor goes alongside with the extinction of the living bacteria and we never get one without the other; the completeness of the process can be accurately gauged by the initial bacterial counts (cf. 0·25 per cent. of CS_2 in Table VIII) and by determinations of the amounts of nitrate subsequently formed. This close relationship is precisely what would be expected if the extinction of the harmful factor is a process of killing, *i.e.* if the harmful factor is a living organism.

§ 21. If, however, we suppose that the harmful factor is some physical or chemical condition which is changed to a beneficial factor by antiseptic vapours we must note (1) that once the soil is penetrated the action is not proportional to the mass of the toluene and is therefore irreversible (this is also demonstrated in the next paragraph in

TABLE VIII. *Effect of varying quantities of antiseptics on the bacterial numbers and rate of ammonia production in soils.*

(*a*) Toluene. Arable soil as before (p. 173, Table VII).

Quantity of antiseptic used per 100 of soil	Millions of bacteria per gram of dry soil		
	At start	After 30 days	After 80 days
None	11	8	9
0·25	2·7	47	52
0·5	2·3	35	44
1·0	2·9	36	57
2·0	2·5	36	45
4·0	2·7	35	43

Richer soil, *RC*, containing 23 % water, 0·37 % N, 0·57 % $CaCO_3$, and losing 11·05 % on ignition.

		After 16 days	After 30 days	After 74 days
None	27·5	10	10	45
0·25	4·0	29	29	91
1·0	3·5	24	26	132
4·0	2·5	31	34	114

(*b*) Carbon disulphide. Richer soil, *RC*.

None	27·5	10	10	45
0·25	9·0	27	17	78
1·0	1·6	17	53	121
4·4	2·3	16	32	92

(*a*) Toluene. Arable soil containing 12 % water.

Quantity of antiseptic used per 100 of soil	N as NH_3, parts per million			N as NH_3 & nitrate, parts per million		
	At start	After 30 days	After 80 days	At start	After 30 days	After 80 days
None	2	4	2	23	25	30
0·25	10	26	27	25	42	44
0·5	7	26	27	24	44	45
1·0	9	25	29	25	40	47
2·0	11	25	27	27	42	45
4·0	9	29	37	23	42	58

Richer soil, *RC*, containing 23 % water.

		After 16 days	After 30 days	After 74 days		After 16 days	After 30 days	After 74 days
None	4	5	3	3	80	105	102	114
0·25	—	35	35	20	—	116	124	157
1·0	—	40	43	38	—	114	117	130
4·0	—	39	43	67	—	123	126	155

(*b*) Carbon disulphide. Soil *RC* containing 23 % water.

None	4	5	3	3	80	105	102	114
0·25	—	29	33	62	—	105	109	140
1·0	—	37	40	77	—	111	114	152
4·0	—	39	44	55	—	114	118	130

another way), (2) that the action is rapid and does not require the addition of energy. It follows for both reasons that the new state is more stable than the old. We shall see that other experiments lead to precisely the opposite conclusion (§ 30). This hypothesis therefore leads to contradictory results.

The biological nature of the detrimental factor.

This is further demonstrated by the experiments recorded in the following sections.

§ 22. *Irreversibility of the extinction process.* When the factor is put out of action it does not reappear. The bacterial numbers in the toluened and heated soils maintain their high level (Tables I and IV). Further, a soil that has once been treated with toluene is not altered by re-treatment after a long interval. Table IX shows the results of experiments in which soil was partially sterilised and kept moist and aerated but free from infection for many months so that active bacterial change might go on; at the end of the period the soil was divided into two parts, one of which was re-treated with toluene while the other was not. The soils were again stored under identical conditions of moisture, aeration, etc. and periodically subjected to analysis.

The immediate effect of the re-treatment is to bring the bacterial numbers down to a comparatively low level as happened after the original treatment. When the toluene is evaporated and the soil moistened the numbers begin to rise, but they only slowly attain their previous level and never much exceed it; the change is thus altogether different from that occurring when an untreated soil is toluened. It will be noticed also that treatment of a previously heated soil has no permanent effect on bacterial numbers, the toluene having done nothing that was not already done by the heat: certain differences have, however, been observed in other soils.

§ 23. The factor is therefore not produced in soils kept free from reinfection; once it is removed it does not reappear. It is therefore neither a bacterial product nor any consequence of bacterial action, for if it were it should accumulate to a recognisable extent in the partially sterilised soils where large numbers of bacteria have been present over long periods.

The new flora compared with the old. The non-bacterial nature of the detrimental factor.

§ 24. The bacterial flora of the partially sterilised soil is simpler than that of the untreated soil, but the missing groups can be introduced

Date of original treatment	Date of re-treatment		Millions of bacteria per gram			N as NH_3, parts per million			N as NH_3 and nitrate, parts per million		
			At time of re-treatment	After 45 days	After 100 days	At time of re-treatment	After 45 days	After 100 days	At time of re-treatment	After 45 days	After 100 days
Aug. 17th, 1908	July 13th, 1910	Soil untreated	—	14·5	15	2	1	1	44	25	80
		Treated with toluene	—	70	57	5	2	2	91	107	112
		Treated with toluene, and then re-treated	—	60	52	5	21	17	91	109	112
		Arable soil as before—									
May 29th, 1909	Oct.* 27th, 1911	Untreated	8	11	8	—	—	1	—	—	84
		Untreated, and then treated with toluene	1·4	53	37	—	—	18	—	—	100
		Treated with toluene	39	43·5	36	—	—	1	—	—	89
		Treated with toluene, and then re-treated	15·4	48	46	—	—	12	—	—	100
		Heated to 98°	36	67	38	—	—	0	—	—	89
		Heated to 98°, and then treated with toluene	11	61	44	—	—	5	—	—	92
				After 28 days	After 98 days		After 28 days	After 98 days		After 28 days	After 98 days
Nov. 4th, 1911	Dec. 21st, 1911	Soil *RC* (see Table VIII)—									
		Untreated	66	60	28	4	3	5	108	111	125
		Treated with toluene	120	118	62	24	7	—	122	123	—
		Treated with toluene, and then re-treated	—	60	30	...	46	49	—	136	141
		Treated with CS_2	110	109	36	44	48	—	127	129	—
		Treated with CS_2, and then re-treated	—	67	60	...	53	50	—	129	131

* The percentage of nitrogen was determined in these soils on Jan. 30th, 1912, *i.e.* 100 days after re-treatment, and was found to be—

Treatment on May 29th, 1909	N °/₀ on Jan. 30th, 1912
Untreated	·152
Heated	·143
Treated with toluene which was then allowed to evaporate	·139
Treated with toluene which was then left in	·145

This relative loss of nitrogen from partially sterilised soils is quite usual (see this *Journal*, 1909, **3**, 126, also 1912, **5**, 38, 98).

by infecting with a little of the untreated soil, or its water extract. Reference to Table X shows that the added bacteria develop side by side with those already present in the toluened soil, and there is nothing to indicate that any antagonism exists between the two groups. Now the added organisms include those killed by toluene; the flora in the infected soil therefore approximates qualitatively to that normally present in the untreated soils. But this infected soil flora is always more numerous than that in the toluened soil, and it follows that the flora setting up after partial sterilisation is less able to attain high numbers than the original flora, other conditions being the same. The high bacterial numbers associated with partially sterilised soils are therefore not the result of any improvement in the bacteria themselves.

§ 25. This conclusion rules out all hypotheses based on the assumption that partial sterilisation causes the bacteria to multiply more rapidly either by imparting some stimulus or by removing certain groups of bacteria which somehow prevent the others from multiplying[1]. It is seen on the contrary that partial sterilisation adversely affects the multiplying power of the bacteria, and the increased numbers follow, not because of the change in type, but in spite of it. The harmful factor is in short associated with something external to the bacteria.

§ 26. Two interesting facts are brought out in Table X. It is clear that the bacteria on the toluened soil do not occupy the whole ground even when they have attained their maximum numbers because there is still room for other organisms. In Exp. I, for example, the

[1] It is sometimes stated that the new flora has a more favourable effect on the accumulation of plant food in the soil than the old because the denitrifying organisms are killed during partial sterilisation. This statement has several times been disproved but is nevertheless constantly reappearing. We have made numerous experiments on the subject and find that the denitrifying organisms, like the rest, increase after partial sterilisation. As examples, two experiments may be quoted in which soil was inoculated into Giltay's solution; the following amounts of nitrate were destroyed:

	Experiment 1			Experiment 2		
	After 24 hours	After 40 hours	After 64 hours	After 20 hours	After 30 hours	After 40 hours
Untreated soil	3·9	13·5	13·0	2·0	4·9	7·2
Soil treated with toluene	8·1	13·5	13·3	5·6	8·6	14·3
Soil heated to 100° C.	0	10·0	13·8	—	—	—
Soil heated to 56° C. for 4 hours	—	—	—	3·1	6·8	12·6

It has already been shown that the rate of loss of nitrogen from the soil (presumably as gas) is greater in partially sterilised than in untreated soils; see footnote. p. 177.

TABLE X. *Effect of introducing untreated soil into partially sterilised soils.*

Millions of bacteria per gram of dry soil.

	Experiment 1				Experiment 2				
	At start	After 40 days	After 103 days	After 160 days	At start	After 28 days	After 110 days	After 200 days	After 320 days
Arable soil—									
Untreated soil	11	16	9	13	—	14	9	12	8
Toluened soil	2·2	43	41	43·5	—	59	71	81	85
Toluened soil + 0·5 % of untreated soil	—	60	71	47	—	85	54	103	64

	Experiment 3		
	At start	After 45 days	After 95 days
Untreated soil	7	11	12
Toluened soil	2·4	52	41
Toluened soil + water extract of untreated soil	2·2	69	94

	Experiment 4 Richer soil, *RC*			Experiment 5 Very rich soil, *OxL*		
	At start	After 21 days	After 115 days	At start	After 15 days	After 115 days
Untreated soil	9	36	19	70	82	45
Toluened soil*	3·6	73	32	—	245	185
Toluened soil + 0·5 % of untreated soil	—	122	70	—	287	222
Toluened soil + water extract of untreated soil	—	131	53	—	409	250

* In Experiments 4 and 5 the action of toluene was incomplete as nitrification began after the 20th day (see p. 205).

The amounts of nitrogen present as ammonia and nitrate on the various dates are given in Table XIV.

toluened soil supports some 43 million bacteria per gram and no more: but as soon as fresh groups are introduced from the untreated soil another 30 million organisms find room. Some interesting questions are thus raised to which we hope to revert on another occasion. The second fact and one we must now consider a little more fully is that the bacterial numbers fall off in the infected soils even where the numbers in the uninfected soils have remained constant. This is seen

clearly in Exps. 1 and 2 but it is masked in Exps. 4 and 5 by a fall in numbers on the toluened soil. The experiment necessitates the introduction into our partially sterilised soils of some of the untreated soil and therefore of any of the harmful factor that might be present.

§ 27. *The introduction of the harmful factor into partially sterilised soil.* A detailed series of experiments was arranged to study the effect of introducing untreated soil containing the harmful factor into partially sterilised soils, the method adopted being to mix known proportions of untreated soil with the partially sterilised soil and make periodical bacteriological analyses. Typical results are shown in Table XI and in Fig. 3, from which it appears as before that the immediate effect of the admixture is to increase the bacterial numbers. In Exp. 1 high numbers are maintained where 0·5 per cent. but not where 5 per cent. of untreated soil is added. In Exp. 2 the numbers are all high for the first 21 days but they subsequently fall off considerably where untreated soil is present, in all but one instance becoming much less than numbers

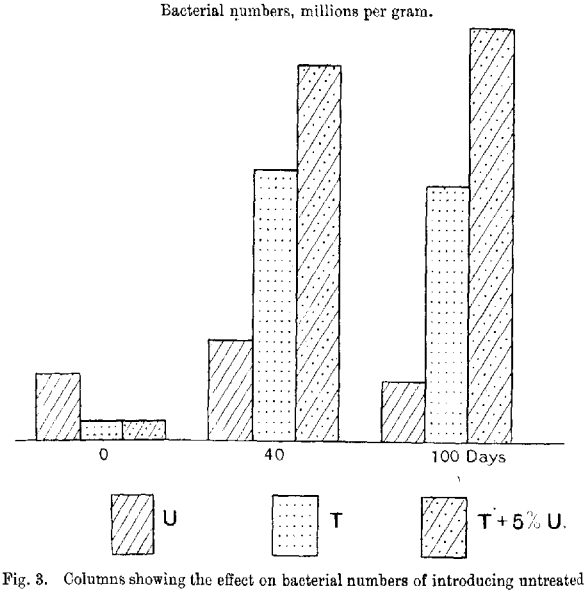


Fig. 3. Columns showing the effect on bacterial numbers of introducing untreated soil into partially sterilised soils. (*a*) 1st period, where an increase is produced through the activity of the added bacteria.

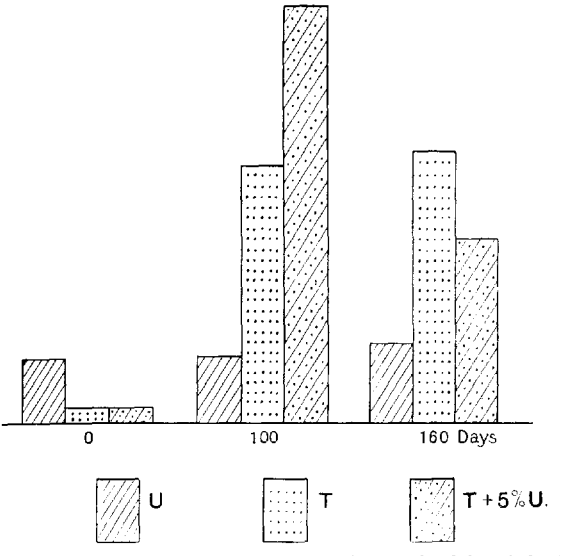


Fig. 3 (*continued*). (*b*) 2nd period, where a depression is produced through the slower development of the harmful organisms (Table XI, Exp. 3).

calculated from the proportions of toluened and untreated soil on the assumption that the soils are inert to one another. In all experiments this divergency is noticed after a longer or shorter period; the final counts in Table XI and the calculated figures are as follows:

Per cent. of untreated soil present		0·5	5	20	50	100
Exp. 1.	Calculated numbers	74	71	—	—	
	Observed	115	61	—	—	14
Exp. 2.	Calculated numbers	44	42	36	25	
	Observed	28	16	29	35	7
Exp. 3.	Calculated numbers	43	41	37	28	
	Observed	47	35	23	16	13
Exp. 4.	Calculated numbers *	—	220	191	—	
	Observed	231	173	148	—	37
Exp. 5.	Calculated numbers	201	192	166	111	
	Observed	74	170	122	28	20

* From toluened soil infected with 0·5 % untreated soil (see note to Exp. 4 on Table XI).

TABLE XI. *Effect on bacterial numbers of introducing untreated soil into partially sterilised soils and vice versa.*

Millions of bacteria per gram of dry soil.

Experiment 1. Arable soil as before.

(*a*) Untreated soil added to partially sterilised soil.

	At start	After 50 days	After 100 days
Toluened soil	2·1	71	74
,, ,, + 0·5 % untreated soil	3·2	103	115
,, ,, + 5 % ,, ,,	4	58	61

(*b*) Partially sterilised soil added to untreated soil.

Untreated soil	8	17	14
,, ,, + 0·5 % toluened soil	8	14	17
,, ,, + 5 % ,, ,,	5	11	14

(*a*) Untreated soil added to partially sterilised soil.

	Exp. 2. Arable soil				Exp. 3. Arable soil			
	At start	After 7 days	After 21 days	After 58 days	At start	After 39 days	After 100 days	After 157 days
Toluened soil	2·9	41	66	44	2	43	41	43
,, ,, + 0·5 % untreated soil	—	76	72	28	—	60	71	47
,, ,, + 5 % ,, ,,	—	118	120	16	—	60	66	35
,, ,, + 20 parts* ,, ,,	—	67	102	29	—	45	15	23
,, ,, + 50 parts* ,, ,,	—	64	61	35	—	18	25	16

(*b*) Partially sterilised soil added to untreated soil.

Untreated soil	10	9	16	7	11	16	9	13
,, ,, + 5 % toluened soil	—	19	16	8	—	23	17	15
,, ,, + 20 parts* ,, ,,	—	22	36	10	—	16	17	17

	Exp. 4. Richer soil			Exp. 5. Very rich soil		
	After 33 days	After 83 days	After 170 days	After 15 days	After 69 days	After 154 days
Toluened soil	103	90	†	152	225	202
,, ,, + 0·5 % untreated soil	97	80	231	233	255	74
,, ,, + 5 % ,, ,,	104	51	173	243	117	170
,, ,, + 20 parts* ,, ,,	123	84	148	267	171	122
,, ,, + 50 parts* ,, ,,	—	—	—	136	74	28
Untreated soil	33	33	37	101	83	20

The initial counts in Exps. 4 and 5 and that marked † were lost through liquefaction of the plates.

The composition of the soils and the amounts of nitrogen present as ammonia and nitrate on the various dates are given in Table XIV.

* Parts per 100 of mixture, *i.e.* 80 of toluened + 20 of untreated soil or *vice versa* and 50 of toluened + 50 of untreated soil; the figures are used in this way throughout the Table.

§ 28. Now we know that these calculated numbers are too low because they take no account of the increase in bacterial numbers that follows introduction of untreated soil into toluened soil, but even so the observed numbers come out still lower. Thus the effect of introducing 5 per cent. or more untreated soil into partially sterilised soil is first to increase and then after a time to considerably reduce the bacterial numbers, in some cases bringing them down near to the level of the untreated soil.

It might be argued that the high bacterial numbers first induced by additions of untreated to partially sterilised soils exhaust the supply of some essential nutrient set free by the toluene and thus inevitably lead to a reduction in numbers. In this way some of the results of Table XI might be explained (*e.g.* Exp. 2, and parts of Exps. 3 and 4) without assuming that any detrimental organisms come into play. On the other hand, Exps. 1, 5, and parts of 3 and 4 cannot be thus explained, and the only hypothesis that covers all the results is that the harmful factor has been transmitted to the partially sterilised soils. We thus have further proof that the factor is something positive and is not a negative state such as lack of a stimulant or essential requirement. The second part of the experiment also affords evidence that the untreated soil is not inert but contains a positive detrimental factor: the addition of as much as 20 per cent. of partially sterilised soil to untreated soil fails to increase the bacterial numbers, excepting temporarily and to a small extent. (See also § 7.) Other examples of depression of bacterial numbers in partially sterilised soils by infecting with untreated soils are given in Table XVII (p. 217). The partially sterilised soil B in that Table was mixed with an equal weight of untreated soil and left for 5½ months. At the end of that time the soils were mixed with hay dust: the bacterial numbers subsequently found were in millions per gram:

	Just before addition of hay	7 days after addition of hay	74 days after addition of hay
Toluened soil	31	175	136
Toluened soil + equal weight of untreated soil	25	86	66
Untreated soil	7	94	62

The mixed soil is now indistinguishable from the untreated soil, and the advantage of partial sterilisation has wholly disappeared.

§ 29. The harmful factor is not invariably transmitted to the same extent from the untreated to the partially sterilised soil and in a few cases indeed it is not transmitted at all. The falling off of bacterial numbers from the calculated values follows no sort of rule, being related neither to the numbers of bacteria nor to the amount of added soil. Indeed we get the same erratic changes as in the untreated soils in Tables I and II. Only rarely is the transmission so complete as to bring the numbers down to the level of the untreated soil.

§ 30. It is possible to explain these results on the supposition that partial sterilisation has effected some change in the soil colloids, making them more favourable for bacterial activity. Changes in surface tension and other properties are almost certain to take place and to react on bacterial activity. But we get into difficulties directly we suppose that this is the sole cause at work. For example: when some of the untreated soil is added the new form reverts to the old and less suitable form; addition of the new form to the old (*i.e.* of 5 per cent. of toluened soil to untreated soil) is, on the contrary, without effect. The new form is therefore less stable than the old at ordinary temperatures. This result appears to be in entire contradiction with one obtained earlier (§ 21). The supposition is also difficult to reconcile with the evidence of the active nature of the factor (§ 7) and we must therefore discard it as a satisfactory explanation of all the phenomena.

§ 31. The bacteriotoxin hypothesis does not account for the results. The depression produced by the introduction of the untreated soil ought to come into operation at once if it were caused by a toxin, and the amount of the depression should be proportional to the amount of added soil. Neither of these results is obtained. Further, as shown in our earlier paper and in Table X, the water extract of an untreated soil has no toxic effect when added to a toluened soil and not infrequently causes an increase in bacterial numbers because it itself carries bacteria. Toxic properties have been attributed to this extract by Greig-Smith[1] and by Bottomley[2], but we are unable to obtain their results with our soils. Greig-Smith worked with soils in New South Wales which are unfortunately inaccessible to us in a fresh condition, so that we are unable to account for the discrepancy[3]. Bottomley used soils from the Chelsea Physic garden, but in these also we failed to find evidence of a toxin by the methods he adopted.

[1] *Transactions of the Linnean Society of New South Wales*, Nov. 30th 1910.

[2] *Report of the British Association*, 1911.

[3] It might arise from a difference in the amount of calcium carbonate present.

III. THE PROPERTIES OF THE INJURIOUS FACTOR AND ITS PROBABLE NATURE.

§ 32. The properties of the injurious factor ascertained by the preceding experiments are as follows:

(*a*) It is permanently put out of action by toluene and other antiseptics sufficiently potent to kill nitrifying organisms, and also by heating to 55°. If the soils are kept free from reinfection it does not reappear even though the conditions are made very favourable for bacterial growth.

(*b*) It is temporarily put out of action by lesser degrees of heat, *e.g.* 50° or less, by drying for a sufficient length of time at 35°—40° and by low temperatures. After a time it manifests itself again if the soil is kept under normal conditions of temperature, water supply and aeration.

(*c*) It can be reintroduced into a soil from which it has been permanently extinguished by the addition of a little untreated soil.

(*d*) It develops more slowly than bacteria and for some time may show little or no effect[1]; then it causes a marked reduction in the

[1] This slow growth of the destructive organisms, which was emphasised in our earlier paper, vitiates some of the criticisms that have been passed on our conclusions. For example, Lipman, Blair, Owen and McLean (Experiments relating to the possible influence of protozoa on ammonification in the soil, *New Jersey Expt. Station Bull.* 248, 1912) added pasteurised and untreated soil infusions respectively to mixtures of sterilised soil (heated under a pressure of 1·5 atmospheres of steam) and dried blood. After seven days the pasteurised infusion had induced the formation of no more ammonia than the untreated infusion. They conclude that these results "do not bear out Russell and Hutchinson's contention as to the part played by protozoa in depressing the activities of soil bacteria."

It does not appear to us that the experiment really bears on the subject. In no case have we observed development of the destructive organisms in anything like so short a time as seven days. Two assumptions are also involved which the facts do not warrant: (1) the amount of ammonia formed is taken as a measure of the number of bacteria (see pp. 191 *et seq.* on this point), (2) subjecting the soil to the high temperature of steam at 1½ atmos. is supposed to leave it unchanged. The argument as we understand it reduces itself to this: the destructive organisms made no growth in seven days in a medium A (strongly heated soil), therefore they could make no growth in a longer period in a wholly different medium B (ordinary unheated soil).

In common with other soil investigators Fred (Über die Beschleunigung der Lebenstätigkeit höherer und niederer Pfanzen durch kleine Giftmengen, *Centr. Bakt. Par.* 1912, II, **31**, 185—245) assumes that heating the soil has no effect except to kill micro-organisms. He heated soil to 100° C., added ammonium sulphate and then ether, and continues "nach Russell und Hutchinson's ansicht würde dieses Antiseptikum in amöbenfreiem Boden dann keine günstige Wirkung haben" (we expressly stated in our earlier paper that we made no such claim; see also p. 156 here). No favourable action was observed, as a

numbers of bacteria and its final effect is out of all proportion to the amount originally introduced. The development is erratic and we have not learnt precisely the conditions under which it best takes place.

(*e*) It is shown not to be bacterial in nature (§§ 23, 25), nor a toxin (§§ 12, 23, 31), nor any adverse physical or chemical state of any of the soil constituents (§§ 21, 30) nor any negative condition such as lack of some essential or desirable factor (§§ 7, 28).

(*f*) It is favoured by conditions favourable to trophic life in the soil[1].

(*g*) We see no escape from the conclusion that it is a living organism.

§ 33. In our previous paper we identified the injurious organisms with the soil protozoa and our subsequent work supports this view. Examination of a large number of soils shows that protozoa are normal inhabitants of the soil. The total number of species present must be considerable. Goodey[2] has examined hay infusion cultures and described a number of forms that he picked out, and Martin[3] has used a plate culture method and got out others. Protozoa commonly and perhaps invariably get into diseased roots wherever many bacteria have entered, but so far as we know no one has investigated them; there can be no doubt that they would amply repay study by some competent zoologist. Protozoa have been found in German soils by Hiltner[4], Störmer[5], Max Wolff[6], and R. H. Francé[7]; in the soils of Hawaii by S. S. Peck[8], and of Porto Rico by Oscar Loew[9]. The evidence seems

matter of fact, excepting only when untreated soils were treated with ether and the author admits that "Diese Beobachtung spricht für Russell und Hutchinson, doch"—he naively continues—"doch ist es möglich, und sogar wahrscheinlich, dass die gesteigerte Nitrifikation durch Äther auf einer Reizwirkung auf die nitrifizierenden Bakterien selbst beruht."

[1] This aspect is discussed in this *Journal*, 1912, **5**, 27, 86.

[2] A contribution to our knowledge of the protozoa of the soil. *Proc. Roy. Soc.* 1911, **84** B, 165—180.

[3] A note on the protozoa from sick soils; *ibid.* 1912, **85** B, 393—400.

[4] Ueber neue Ergebnisse und Probleme auf dem Gebiete der landwirtschaftlichen Bakteriologie, *Jahresber. Verein für Angew. Botanik*, 1907, **5**, 200.

[5] Die Wirkung des Schwefelkohlenstoffs auf dem Boden, *ibid.* p. 123.

[6] Der Einfluss der Bewässerung auf die Fauna der Ackerkrume mit besonderer Berücksichtigung der Bodenprotozoen, *Mitt. Kaiser Wilhelm Instit. für Landw.*, Bromberg, 1909, **1**, 382—401; Ueber Bodenprotozoen, *Centr. Bakt. Par.* 1912, II, **33**, 314—320.

[7] Studien über edaphische Organismen, *Centr. Bakt. Par.* 1912, II, **32**, 1—7.

[8] Some Bio-chemical investigations of Hawaiian Soils, *Bull.* 34, *Expt. Station of the Hawaiian Sugar Planters' Association*, 1910.

[9] *Annual Report of the Porto Rico Agricultural Expt. Station*, 1910. Also R. Emmerich, W. Graf zu Leiningen u. O. Loew, Über Bodensaüberung, *Centr. Bakt. Par.* 1912, II, **31**, 466—477. Other references are given in Goodey's paper (*loc. cit.*).

to be conclusive that any of these organisms occurring in an active state would be inimical to bacteria and would therefore function as the injurious factor.

Rahn[1] has not only found protozoa in Michigan soils but has formed a minimum estimate of the numbers present per gram, arriving at results of the same order as our own. He further demonstrated that the numbers are considerably reduced on drying.

The great difficulty, and one that none of the seven investigators just mentioned has attempted to deal with, is to determine which forms are active and which remain as cysts under the conditions of the soil. Goodey has devoted much attention to the ciliates developing in hay infusion (Colpoda, etc.) but could find no evidence that they are active in the soil. Martin, on the other hand, considers that some of his organisms—amoebae and amoeboid forms—probably are active. Some of our observations are difficult to explain except on the view that certain protozoa are capable of growth; thus it has happened when small quantities of untreated soil have been added to toluened soil that during the first few days we failed to find either ciliates or amoebae, while later on we found them without difficulty. It is difficult to see why protozoan cysts should remain undeveloped in the soil; bacterial spores certainly show no tendency to accumulate and rarely form more than 20 or 30 per cent. of the total numbers growing on gelatine (Tables VIII, X and XI). The problem, however, is not likely to be solved until the zoological survey of the soil has proceeded further, and accurate methods devised for counting the protozoa.

§ 34. The examination of soil for protozoa is now part of our routine procedure in all experiments on partial sterilisation. Soil is inoculated into a one per cent. hay infusion and left in an incubator at 25° for 4—5 days, examination being made periodically for protozoa. No identification is attempted, but the organisms are grouped roughly as ciliates, amoebae, and monads; no attempt is made even approximately to estimate their numbers. Partial sterilisation invariably simplifies the fauna considerably, killing the ciliates and amoebae but often leaving certain monads. *Whenever the ciliates and amoebae are killed we invariably find that the detrimental factor is extinguished; whenever the detrimental factor is not extinguished the protozoa also are not killed;* we have found no exception to these rules.

[1] Methode zur Schätzung der Anzahl von Protozoën im Boden. *Centr. Bakt. Par.* 1913, II, **36**, 419—421.

The following are typical results obtained for heated soils:

	Bacteria after 68 days. Millions per gram of dry soil	NH_3 and nitrate formed after 68 days. Parts per million of soil	Detrimental factor	Protozoa found
Untreated soil	11·1	13	present	Ciliates, Amoebae, Monads
Heated to 40° for 3 hours	7·5	11·4	present	Ciliates, Amoebae, Monads
Heated to 56° for 3 hours	37·5	36·7	killed	All killed

Treatment with toluene leads to similar results:

	Bacteria after 30 days. Millions per gram	NH_3 and nitrate formed after 30 days	Detrimental factor	Protozoa found
Untreated soil	8	24·5	present	Ciliates, Amoebae, Monads
Toluened soil	47·4	41·6	killed	All killed but certain Monads

Quicklime also produces the same effect. So long as small quantities only are added the protozoa are not appreciably affected, the bacterial numbers do not fall in the beginning nor show any subsequent rise. But when a certain larger quantity has been added the partial sterilisation effect is produced, the protozoan fauna is considerably simplified and the bacterial numbers are at first depressed but later on they rise considerably and effect a corresponding production of ammonia (see § 18).

In Table XII (p. 194) it is shown that a soil stored moist in a closed bottle for 37 years still retained a complex protozoan fauna and a low bacterial population. But after treatment with toluene the protozoa were destroyed (excepting certain Monads) and the bacterial population rose considerably.

But when the soil was heated insufficiently to kill the harmful factor more of the protozoa survive:

	Detrimental factor	After 13 days		After 53 days		After 245 days	
		Bacteria millions	Protozoa	Bacteria millions	Protozoa	Bacteria millions	Protozoa
Untreated ...	Present	8	C. A. M.*	9	C. A. M.	12	C. A. M.
Heated to 50° for 1 hour...	Present but suppressed	26	C. A. M.	26	M.	16	C. A. M.
Heated to 50° for 24 hours	Temporarily extinguished	15	none	16	?	20	A. M.

* The contractions C. A. M. stand respectively for Ciliates, Amoebae and Monads.

	At start		After 16 days		After 30 days		After 74 days	
	Bacteria millions	Protozoa	Bacteria millions	Protozoa	Bacteria millions	Protozoa	Bacteria millions	Protozoa
Untreated	27·5	C. A. M.	10	C. A. M.	10	C. A. M.	45	C. A. M.
0·3 % CS_2	9	A. M.	27	C. M.	17	C. M.	78	C. A. M.
1·0 % CS_2	1·6	M.?	17	M.	53	M.	121	A. M.

With a still richer soil even more marked results are obtained. In this case carbon disulphide so effectively reduced the protozoa that we could not be sure that any were left, while toluene was much less destructive. The subsequent rise in bacterial numbers was considerably higher after treatment with carbon disulphide than with toluene. The fall in numbers on adding 5 per cent. of the untreated soil is also seen to be accompanied by an appearance of a complex protozoan fauna.

	After 83 days	
	Bacteria millions	Protozoa
Untreated	90	C. A. M.
Treated with CS_2	326	None found
,, ,, ,, then mixed with 5 % untreated soil	155	C. A. M.
Treated with toluene	106	C. A. M.

§ 35. In several of the above instances there is evidence of an increasing complexity in the fauna as time goes on, and the simplest explanation is that there has been an actual multiplication of some of the forms which at first were present in numbers too small for us to detect. But we cannot lay too much stress on this point, as the organisms multiply rapidly in hay infusions and we are unable in our final examinations to say whether the soil we started with contained a large or a small number of organisms. This indeed is the weakness of the method. But this very weakness only makes the close connection between the destruction of the protozoa and the destruction of the harmful factor all the more striking. The survival of protozoa in other experiments unfortunately loses much of its significance because it may only mean that a small number of cysts escaped, but even here it will be noticed that the highest bacterial numbers are never attained when the fauna is complex, *i.e.* when a relatively large number of protozoa survive. In rich soils toluene often fails to kill all protozoa just as it fails to kill nitrifying organisms and to cure "sickness"; this has been traced to its low solubility and consequent inability to penetrate any but small particles of soil in presence of much moisture or organic matter[1].

On the other hand, we have failed in our attempts to reduce bacterial numbers in a partially sterilised soil by introducing mass cultures of the ordinary hay infusion protozoa. Our difficulty has been to remove from the cultures the large contaminations of bacteria and bacterial food which cause disturbances directly they get into the soil; we never have a really clean experiment.

§ 36. Until a more complete zoological survey of the soil has been made it is not possible to identify the harmful organisms with certainty. We do not even know how they act; whether they devour the bacteria or whether they are present as films round the minute particles of organic matter that would otherwise serve as bacterial food, thus starving the bacterial population down to low limits. The present position in fact, is precisely that in which nitrification stood for many years; the process was known to be biological as far back as 1879, but the most diligent search among the colonies on the gelatine plate cultures then in vogue failed to bring out the organism. Not till 1891, when a new method was devised, could the organism be isolated with certainty. Our present methods of dealing with soil protozoa are those devised for dealing with pond and stream protozoa, and do not precisely

[1] See Russell and Petherbridge, this *Journal*, 1912, **5**, 107.

reproduce the conditions obtaining in the soil. Therefore we must not at this stage lay too much stress on any relationship that comes out, but may only be accidental, between our detrimental organisms and any of the ciliates, amoebae and monads that these methods reveal. But it seems safe to draw two conclusions: (1) the detrimental organisms possess the properties of protozoa and not of bacteria; (2) the presence or absence of the detrimental organisms is intimately associated with the presence or absence of a complex protozoan fauna. We shall therefore continue to identify the detrimental organisms with the soil protozoa without, however, committing ourselves to any particular organism or set of organisms or to any rigid and exclusive definition of the term protozoa.

IV. THE RELATIONSHIP BETWEEN THE RATE OF PRODUCTION OF PLANT FOOD IN THE SOIL AND THE INCREASED NUMBERS OF BACTERIA BROUGHT ABOUT BY PARTIAL STERILISATION.

§ 37. *Partial sterilisation by volatile antiseptics.* It was shown in our earlier paper that the increases in bacterial numbers brought about by partial sterilisation with volatile antiseptics lead to corresponding increases in the amount of ammonia produced in the soil. Subsequent experiments have shown that this relationship does not hold universally, but ceases to manifest itself as soon as a certain amount of ammonia and nitrate has accumulated. There is a fairly well-marked limit beyond which the accumulation of ammonia and nitrate will not go, although bacterial multiplication may still continue. This limiting amount varies for different soils and is higher for soils rich in organic matter and therefore capable of retaining a considerable amount of water than for poor soils of lower water-holding capacity; it is also higher in heated than in unheated soils.

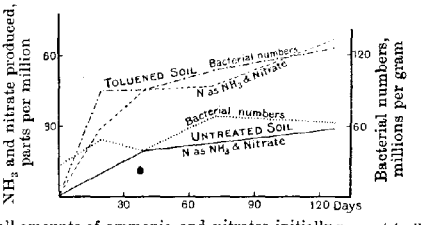
§ 38. Determinations of ammonia and nitrates[1] have been made in all our soils on each occasion when the bacterial numbers were estimated, and the whole of the large mass of data thus obtained can be grouped into two cases:

(1) Soils where the ammonia and nitrates fall well below the limit; here the increase in bacterial numbers following on partial sterilisation causes a corresponding increase in the amount of ammonia and nitrate.

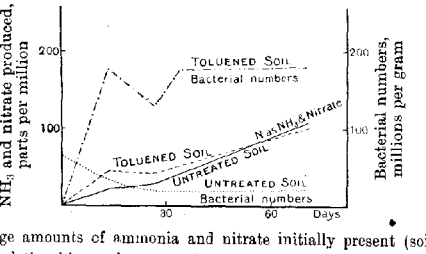
(2) Soils containing much ammonia and nitrate; here the increased numbers of bacteria cause no corresponding increase in the amounts of ammonia and nitrate.

[1] See footnotes to Tables V and VI (pp. 168 and 169).

These cases are illustrated by the curves in Figs. 4 and 5 showing the amounts of ammonia and nitrate, and also the numbers of bacteria present after certain intervals of time. In the diagrams illustrating Case 1 there is a sufficiently close agreement between the curves for bacterial numbers and those for the amounts of ammonia and nitrate to show the intimate relationship between these quantities. Here the highest amount of ammonia present is 45 parts per million in the



Case 1. Small amounts of ammonia and nitrates initially present (soil *RC*, Table XII (*a*)). A relationship is indicated between the bacterial numbers and the rate of accumulation of N as NH_3 and nitrate.



Case 2. Large amounts of ammonia and nitrate initially present (soil *OxL*, Table XII). No such relationship can be seen as in Case 1.

Fig. 4. Ammonia and nitrate produced after certain intervals of time, and also the numbers of bacteria present per gram of soil.

toluened soil, while the ammonia and nitrate finally amount to 150 parts per million. On the other hand, in the diagrams illustrating Case 2 there is no similarity whatsoever between the curves for bacterial numbers and the corresponding curves for the amount of ammonia and nitrate present. The production of ammonia and nitrate, in fact, proceeds at the same rate in both soils, the initial advantage gained by the toluened soil never being improved upon in spite of the large difference in bacterial numbers. But in this soil the amount of

ammonia is 180 parts per million, while the ammonia + nitrate is over 500 parts per million.

Further instances are given in Table XII.

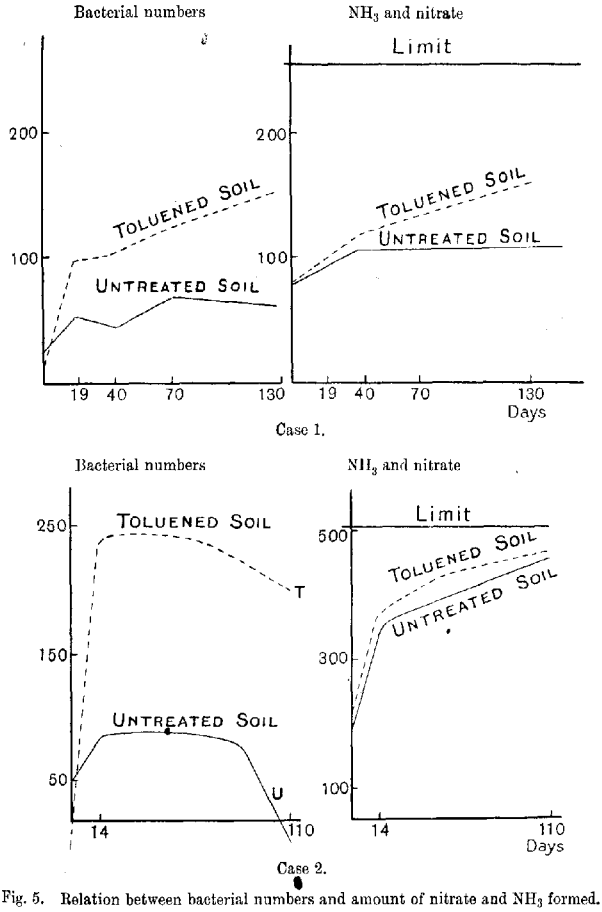


Fig. 5. Relation between bacterial numbers and amount of nitrate and NH_3 formed.

§ 39. The falling off in the rate of accumulation of ammonia and nitrate is due to the ammonia and to a less extent to the nitrate already there, and not to the exhaustion of the complex precursors of ammonia

TABLE XII. *Bacterial numbers, and amounts of ammonia and nitrate in soils partially sterilised by volatile antiseptics.*

Case 1. Ammonia and nitrate in relatively low proportions at first, so that a relation can be seen with the bacterial numbers.

(*a*) Soil *RC* containing 23 °/₀ moisture, 0·37 °/₀ N, 0·5 °/₀ $CaCO_3$ and losing 11·0 °/₀ on ignition.

	Bacterial numbers, millions per gram of soil					N as NH_3, parts per million of soil					N as NH_3+nitrate, parts per million of soil				
	At start	After 19 days	After 40 days	After 72 days	After 128 days	At start	After 19 days	After 40 days	After 72 days	After 128 days	At start	After 19 days	After 40 days	After 72 days	After 128 days
Untreated	25	49	41	69	63	4	2	3	3	3	87	97	107	110	117
Treated with toluene	3	89	91	108	143	6	33	45	38	2	81	108	124	128	149

(*b*) Same soil, 23 °/₀ moisture.

	Bacterial numbers, millions per gram				N as NH_3, parts per million of soil				N as NH_3+nitrate, parts per million of soil			
	At start	After 16 days	After 30 days	After 74 days	At start	After 16 days	After 30 days	After 74 days	At start	After 16 days	After 30 days	After 74 days
Untreated	27	10	10	45	4	5	3	3	80	105	102	114
Treated with toluene...	4	29	29	91	7	35	35	20	—	116	124	157
,, ,, CS_2	9	27	17	78	6	29	33	62	—	105	109	140

(*c*) Arable soil containing 14 °/₀ moisture, 0·18 °/₀ N, 3·16 °/₀ $CaCO_3$, and losing 4·6 °/₀ on ignition.

	Bacterial numbers, millions per gram			N as NH_3, parts per million of soil			N as NH_3+nitrate, parts per million of soil		
	After 5 days	After 27 days	After 57 days	After 5 days	After 27 days	After 57 days	After 5 days	After 27 days	After 57 days
Untreated stored at 5°—12°	11	9	7	1	2	1	26	27	27
,, ,, 20° ...	8	5	6	2	1	1	24	32	31
Toluened ,, 5°—12°	8	27	28	13	19	23	32	43	45
,, ,, 20° ...	50	30	30	15	18	7	39	44	[illegible]

TABLE XII—*Continued.*

Case 2. Ammonia and nitrate in high proportions so that no relation can be seen with bacterial numbers.

(*a*) Soil *OxL* containing 40 % moisture, 0·67 % N, 1·9 % $CaCO_3$ and losing 17 % on ignition.

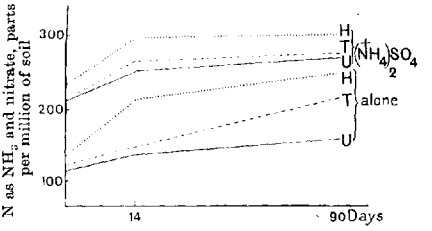
	Bacterial numbers, millions per gram of soil					N as NH_3, parts per million of soil					N as NH_3 + nitrate, parts per million of soil				
	At start	After 13 days	After 25 days	After 70 days	Gain in 70 days	At start	After 13 days	After 25 days	After 70 days	Gain in 70 days	At start	After 13 days	After 25 days	After 70 days	Gain in 70 days
Untreated stored at 5°—12°	65	63	41	32	− 33	14	25	22	18	4	372	391	390	441	69
,, ,, 20° ...	65	41	22	23	− 42	14	23	22	16	2	372	393	397	482	110
Toluened ,, 5°—12°	8	73	101	137	129	87	94	101	197	116	428	439	438	543	115
,, ,, 20° ...	8	187	128	182	174	81	130	123	180	99	428	473	462	530	102

A relationship is indicated at 5°—12° but not at 20°.

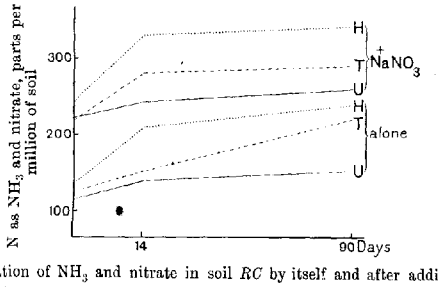
(*b*) Soil from Agdell field bottled moist in 1874, left unopened till November 1911, *i.e.* during 37 years, then divided into three portions. Moisture on opening bottle = 11·6 %.

	Bacterial numbers, millions per gram				Protozoa after 33 days	N as NH_3		N as NH_3 + nitrate	
	At start	After 33 days	After 46 days	After 125 days		At start	After 125 days	At start	After 125 days
Untreated	3	4	5	8	C. M.	0	0	203	207
Treated with toluene ...	3	15	20	38	M.	0	7	200	200
,, ,, CS_2	2	61	46	62	M.	0	6	200	208

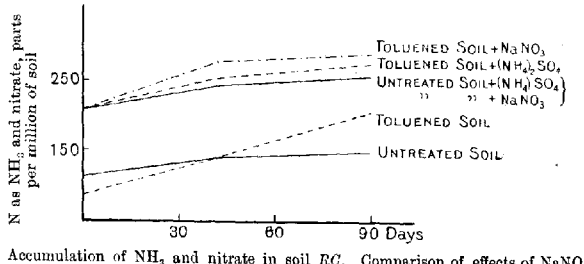
in the soil, since it can be brought about simply by adding suitable quantities of ammonium sulphate and sodium nitrate. The results of such an experiment are given in Table XIII and plotted in Fig. 6, showing the amounts of ammonia and nitrate formed in the soils of



Accumulation of NH_3 and nitrate in soil *RC* by itself and after addition of ammonium sulphate.



Accumulation of NH_3 and nitrate in soil *RC* by itself and after addition of $NaNO_3$ (Table XIII, 1).



Accumulation of NH_3 and nitrate in soil *RC*. Comparison of effects of $NaNO_3$ and $(NH_4)_2SO_4$.

Fig. 6. Effect of additions of ammonium sulphate and sodium nitrate on the rate of accumulation of ammonia and nitrate in soils (Table XIII).

Fig. 4 in presence of ammonium sulphate or sodium nitrate. The limiting effect on the decomposition is well seen. The soil *RC* by itself behaves in the normal way, ammonia and nitrate accumulating on the toluened more rapidly than on the untreated soil. After addition of ammonium sulphate, however, the accumulation no longer goes on so quickly in the toluened soil, and the difference between the curves for this and the untreated soil becomes very small. Sodium nitrate also has an effect but not as great as that of ammonium sulphate. The untreated soil (which is poorer in ammonia and nitrate) behaves differently; neither the addition of ammonium sulphate nor of sodium nitrate has reduced the rate of decomposition. But even at the end of the period the total amount of ammonia and nitrate in this soil still remains below the quantity present in the toluened soils.

§ 40. In soil *OxL* a similar result is obtained. The rate of production of ammonia is at first much higher in the toluened than in the untreated soil. After addition of ammonium sulphate, however, the difference in the rates is considerably reduced, the toluened soil + ammonium sulphate having in six weeks gained little more than the untreated soil + ammonium sulphate. Addition of sodium nitrate appears to be without effect. After the first period of six weeks a drop is observed in the amounts of ammonia and nitrate in the toluened soil and the toluened soil + ammonium sulphate, but not in the other soils. This drop is unusual, and we have rarely observed it in our numerous experiments[1]; the usual course is for the ammonia and nitrate either to remain unaltered or to increase.

§ 41. Two causes may account for the limiting effect exerted by the added ammonium sulphate and sodium nitrate. We may suppose, as Bréal[2] did, that the production of ammonia still continues but a reverse action sets in as soon as the amount of ammonia and nitrate reaches a certain limit, and assimilation of ammonia then takes place. In the numerous cases where the amounts of ammonia

[1] We attribute it to loss of ammonia by volatilisation because we only get it where much ammonia is present and where there has been a distinct loss of water by volatilisation. Thus in the present instance the toluened soil and the toluened soil + ammonium sulphate contained initially 41·5 % and 43·6 % of water, whilst at the end they contained 37·4 % and 35·2 %, losses of 4·1 % and 8·4 % respectively. The amounts of ammonia present reached the unusually high figures of 134 and 277 parts per million (nitrification being considerably retarded) and the falling off from the usual straight line on the curve (*i.e.* the amount we suppose to be lost) to 37 and 49 parts per million.

[2] *Annales Agronomique*, 1896, **22**, 449. Bréal had observed that the production of ammonia went on when the nitrification was suspended by treatment with antiseptics, but soon came to an end. He explained this by assuming assimilation.

TABLE XIII. *Amounts of ammonia and nitrate produced in presence of ammonium sulphate and sodium nitrate respectively.*

(1) Soil *RC*, containing 25 °/₀ moisture, 0·37 °/₀ N, 0·57 °/₀ $CaCO_3$ and losing 11·05 °/₀ on ignition.

	NH_3 and nitrate present in—						NH_3 and nitrate produced		
	Untreated soil		Soil that received $(NH_4)_2SO_4$		Soil that received $NaNO_3$		without addition of $(NH_4)_2SO_4$ or $NaNO_3$	in presence of $(NH_4)_2SO_4$	in presence of $NaNO_3$
	N as NH_3	N as NH_3 + nitrate	as NH_3	as NH_3 + nitrate	N as NH_3	as NH_3 + nitrate			
Untreated soil at start.....	5	113	88	210	5·5	220			
,, ,, after 43 days	3·5	139	3	248	4	244	26	38	24
,, ,, ,, 92 ,,	7	156	4·5	263	5	263	43	53	43
Toluened soil at start	(10)	87	113	212	11	218			
,, ,, after 43 days	59	144	145	257	66	282	57	45	64
,, ,, ,, 92 ,,	90	214	165	275	91	292	127	63	74
Heated soil at start	(30)	136	114	227	30	245			
,, ,, after 43 days ...	97	207	169	291	107	337	71	64	82
,, ,, ,, 92 ,, ...	7	241	163	296	68	346	105	69	101

TABLE XIII—*Continued.*

(2) Soil *OxL*, containing 41 % moisture, 0·63 % N, 1·9 % $CaCO_3$ and losing 17 % on ignition.

	Ammonia and nitrate present in						NH_3 and nitrate produced		
	Untreated soil		Soil that received $(NH_4)_2SO_4$		Soil that received $NaNO_3$		without addition of $(NH_4)_2SO_4$ or $NaNO_3$	in presence of $(NH_4)_2SO_4$	in presence of $NaNO_3$
	N as NH_3	N as NH_3 + nitrate	N as NH_3	as NH_3 + nitrate	N as NH_3	as NH_3 + nitrate			
Untreated soil at start	13	328	118	443	14	467			
,, ,, after 43 days	21	368	21	540	21	506	40	97	39
,, ,, ,, 85 ,,	14	410	14	538	16	516	82	95	49
Toluened soil at start	23	318	171	475	26	459			
,, ,, after 43 days	134	459	277	599	127	589	141	124	130
,, ,, ,, 85 ,,	48	422	194	550	67	601	104†	75†	142
Heated soil at start	64	387	195	515	64	525			
,, ,, after 43 days* ...	255	597	391	721	232	693	210	206	168
,, ,, ,, 85 ,, ...	382	698	414	730	300	769	311	215	244

* By this time there was a copious growth of mould on this soil.

† See footnote [1] p. 197. The 43 days results are probably more reliable than these.

and nitrate remain constant we have further to suppose that the assimilation proceeds at the same rate as the ammonia production, so that only the excess over and above a certain quantity is assimilated. It is also necessary to assume an assimilation of nitrates on the same lines.

The simpler and more probable view is that ammonia production ceases in the soils as soon as a certain amount of ammonia and nitrate is present, the large quantity of ammonia and of soluble nitrates operating as a limiting factor and stopping ammonia production but not necessarily bacterial multiplication. The shape of the curves strongly supports this view, which is further in better accordance with the general nature of biochemical changes. We have also adduced evidence against the assimilation hypothesis in our earlier paper.

§ 42. Table XIV shows that the further increase in numbers on the reintroduction of the original flora into the partially sterilised soil is frequently accompanied by further increases in the amount of ammonia and nitrate produced, but the rule is by no means universal. Whenever the amount of ammonia is high and that of ammonia + nitrate is getting towards the limit, there is a tendency for the rule to be broken.

§ 43. *Partial sterilisation by heat.* The problem presented by heated soils is complicated by at least three disturbing factors. In the first instance heat effects a far more drastic reduction in the bacterial flora than toluene, so that the flora on a heated soil is much simpler than that on a toluened soil. Secondly, it causes a certain amount of decomposition of the organic matter, as is proved by the liberation of ammonia and the dark colour of the aqueous extract. This decomposition lightens the subsequent work of the bacteria with the result that the amount of ammonia and nitrate ultimately produced is much higher than when the partial sterilisation has been effected by antiseptics. Lastly, some of the decomposition products (we have not yet ascertained which) have a toxic action on bacteria so that multiplication does not go on as rapidly as in soils treated with toluene.

§ 44. We do not propose to discuss these effects in detail. Their operation is seen in Table XV where the results of some of our experiments are set out. In 1 the maximum numbers of bacteria are found in the soil that has been heated to 65°, the lowest temperature above that at which the detrimental organisms are destroyed and bacteria can begin to multiply. The numbers are lower in soil heated to 75° and a little lower in that heated to 85°, but very distinctly lower after

heating to 100°, where in fact they are little if any above those in the untreated soil. Similar results are shown by 2, indeed in all the cases so far examined we find maximum bacterial numbers in those soils that have been heated to the minimum temperature necessary to kill the detrimental organisms. Two causes seem to be at work. At this minimum temperature the extermination of the various species is less complete, and we have already seen (§ 26) that a mixed flora can occupy the ground more fully than a simpler one and so attain higher numbers. Also the toxic decomposition products are less in evidence. Soils heated to these minimum temperatures, in fact, closely resemble those treated with volatile antiseptics, since in both cases the secondary disturbances are at a minimum.

§ 45. But if the bacterial numbers are at a minimum in the soils heated to 100° the decomposition effected is at a maximum. There are only a few exceptions to this rule when the amounts of ammonia and nitrate are taken as the measure of the decomposition, and none when the amount of nitrogen assimilated by the plant is taken.

§ 46. The conclusion to which our experiments lead is that a relationship can generally be traced between the bacterial numbers and amount of decomposition in soils that have been heated to 55°—60° (this being the minimum necessary for killing the destructive organisms), just as it can in soils treated with volatile antiseptics; the same limitations also hold in both cases. But no such relationship exists in soils that have been heated to 100°.

Drying the soil has the same effect as heating to low temperatures (Tables VI and XV).

The re-establishment of the original flora, and the introduction of the detrimental organisms.

§ 47. The results of typical experiments on this subject are given in Table XIV. In (1), an arable soil was used containing initially much less ammonia and nitrate (8 parts) than the soil can stand (over 60 parts). In accordance with the general rule we find that the increase in bacterial numbers brought about by partial sterilisation is accompanied by an increase in the amount of ammonia and nitrate in spite of the accumulation of ammonia (see § 38): while the increase in numbers brought about by the addition of bacteria from the untreated soil leads to still further production of ammonia and nitrate. (The ammonia, it should be noted, disappears during the process.)

§ 48. The falling off in bacterial numbers in the infected soils is accompanied by a falling off in the production of ammonia and

TABLE XIV. *Bacterial numbers and amounts of ammonia and nitrate in partially sterilised soils reinfected with the bacterial flora of the untreated soils.*

(1) Arable soil containing 17 % water, 0·18 % N, 3·16 % $CaCO_3$ and losing 4·6 % on ignition.

Mixture containing		Bacterial numbers, millions per gram of soil after				N as NH_3, parts per million of dry soil after				N as NH_3 + nitrate, parts per million of dry soil after			
Untreated soil	Toluened soil	38 days	101 days	158 days	266 days	38 days	101 days	158 days	266 days	38 days	101 days	158 days	266 days
100	0	16	9	13	6	2	1	1	1	8	14	22	46
95	5	23	17	15	9	2	1	1	1	13	17	22	42
80	20	16	17	17	8	2	1	1	1	14	21	26	46
50	50	18	25	16	11	2	1	1	0	26	29	38	52
20	80	45	15	23	11	2	1	1	0	26	37	42	55
5	95	60	66	34	18	10	1	1	1	26	42	49	65
0·5	99·5	60	71	46	25	19	5	2	1	26	43	50	62
0	100	43	41	43	18*	20	27	28	18*	27	34	34	50*

* Infection apparently took place between the 158th and 266th day, as the nitrates, which had remained constant at 7 parts per million, rose during this period to 32 parts.

TABLE XIV—*Continued.*

(2) The same arable soil containing 15 °/₀ water.

	After 4 months		
	Bacterial numbers, millions per gram	N as NH_3, parts per million	N as NH_3 + nitrate, parts per million
Untreated soil	7	3	27
Toluened soil	31	25	43
,, ,, + water extract of untreated soil	50	2	58
,, ,, + 5 °/₀ of untreated soil	45	23	45

(3) The same arable soil containing 12 °/₀ water.

	Bacterial numbers, millions per gram of soil				N as NH_3, parts per million of dry soil				N as NH_3 + nitrate, parts per million of dry soil			
	After 28 days	After 111 days	After 300 days	After 420 days	After 28 days	After 111 days	After 300 days	After 420 days	After 28 days	After 111 days	After 300 days	After 420 days
Untreated soil	14	9	12	8	1	1	2	4·5	34	39	46	48
Toluened soil	59	71	81	85	27	33	35	36	37	50	49	57
,, ,, + 0·5 °/₀ of untreated soil	85	54	103	64	22	36	39	36	30	50	56	52
,, ,, + 5 °/₀ ,, ,,	97	52	75	58	22	32	36	40	33	45	54	58

TABLE XIV—*Continued.*

(4) Soil *SB*, containing 16 % moisture, 0·22 % N, 0·63 % $CaCO_3$, and losing 6·0 % on ignition.

	Bacterial numbers, millions per gram of dry soil			N as NH_3, parts per million of soil			N as NH_3 and nitrates, parts per million of soil		
	At start	After 21 days	After 113 days	At start	After 21 days	After 113 days	At start	After 21 days	After 113 days
Untreated soil	9	36	19	7	3	4	93	122	145
Toluened soil*	4	73	33	7	20	4	95	121	154
,, ,, + water extract of untreated soil	4	131	53	7	19	4	95	120	155
,, ,, + 0·5 % of untreated soil (p. 179)	4	122	70	7	23	4	95	116	151

* Action incomplete, nitrifying organisms not being killed and nitrification only temporarily checked.

(5) Soil *SB*, containing 19 % moisture.

Mixture containing		Bacterial numbers, millions per gram			N as NH_3, parts per million				N as NH_3 and nitrate, parts per million of dry soil			
Untreated soil	Toluened soil	After 33 days	After 82 days	After 168 days	At start	After 33 days	After 82 days	After 168 days	At start	After 33 days	After 82 days	After 168 days
100	0	33	33	37	13	4	2	4	96	92	97	130
20	80	123	83	148	10	14	0	1	94	132	89	161
5	95	104	51	173	9	19	2	2	93	121	164	169
0·5	99·5	97	80	231	9	31	1	2	93	117	147	162
water extract	100	94	51	202	9	38	2	6	93	123	159	145
0	100*	103	90	—	9	43	2	—	93	134	118	—

* Action incomplete, nitrifying organisms not being killed and nitrification only temporarily checked.

TABLE XIV—*Continued.*

6) Rich soil *B*, containing 30 % water. The air-dried soil contained 0·72 % N, 0·92 % $CaCO_3$, and losing 25·0 % on ignition.

Mixture containing		Bacterial numbers, millions per gram			N as NH_3, parts per million of dry soil			N as NH_3 and nitrate, parts per million of dry soil		
Untreated soil	Toluened Soil	After 15 days	After 69 days	After 154 days	After 15 days	After 69 days	After 154 days	After 15 days	After 154 days	Calculated amount* after 154 days
100	0	101	83	20	22	8	13	96	178	
50	50	136	74	28	60	66	9	141	220	239
20	80	267	171	122	75	55	4	160	272	276
5	95	243	117	170	119	40	16	176	324	292
0·5	99·5	233	255	74	86	63	205	162	270	298
water extract	100	230	204	192	129	131	89	174	299	
0	100	152	225	202	137	149	173	205	260	

* Calculated from the values for the untreated soil, and the toluened soil + water extract of untreated soil (*i.e.* toluened soil + bacteria of untreated soil, but with minimum accompaniment of detrimental organisms).

(7) Soil *OxL*, containing 46 % moisture, 0·63 % N, 1·9 % $CaCO_3$ and losing 17 % on ignition.

	Bacterial numbers, millions per gram			N as NH_3, parts per million of dry soil			N as NH_3 and nitrate, parts per million of dry soil		
	At start	After 15 days	After 113 days	At start	After 15 days	After 113 days	At start	After 15 days	After 113 days
Untreated soil	71	82	45	16	17	18	191	360	455
Toluened soil*	146	245	185	35	99	99	225	381	465
,, ,, + water extract of untreated soil	146	408	250	35	101	16	225	404	449
,, ,, + 0·5 % untreated soil (p. 179)	146	287	222	35	107	10	225	398	†

* Action incomplete, nitrifying organisms not being killed and nitrification only temporarily checked. † Lost.

TABLE XV. *Bacterial numbers, and amounts of ammonia and nitrate in soils partially sterilised by heat* and by drying.*

1. Arable soil as before containing 14 °/₀ water.

Temperature to which soil is heated	Bacterial numbers, millions per gram					N as NH_3, parts per million						N as NH_3 and nitrate, parts per million					
	Soon after starting	After 15 days	After 120 days	After 178 days	After 205 days	At start	After 15 days	After 120 days	After 178 days	After 205 days	After 319 days	At start	After 15 days	After 120 days	After 178 days	After 205 days	After 319 days
—	11	—	—	—	—	2	1	1	—	—	—	34	34	39	—	—	—
45°	13	9	4	9	12	2	2	2	10†	2	—	—	35	49	57	53	—
52°	15	11	9	13	23	3	11	1	18†	3	—	—	40	57	75	65	73
55°	—	5	3	13	73	—	15	14	6	4	3	—	42	69	72	72	69
65°	13	21	37	45	60	7	16	21	24	33	30	—	42	65	59	66	63
75°	2	12	32	26	45	—	22	33	36	41	40	—	49	64	64	75	76
85°	4	10	37	14	44	6	23	32	46	36	42	—	47	61	75	63	78
100°	1·4	3	18	8	14	6	22	44	46	55	51	—	49	69	75	79	81

* The soils were heated for 3 hours in the bottles in which they were stored.

† We cannot account for these abnormally high ammonia results, which would usually only be about 2 parts per million. The values for ammonia and nitrate are correspondingly high.

2. Arable soil as before containing 13 °/₀ water.

Temperature to which soil is heated	Bacterial numbers, millions per gram					N as NH_3, parts per million					N as NH_3 and nitrate, parts per million				
	At start	After 7 days	After 21 days	After 68 days	After 142 days	At start	After 7 days	After 21 days	After 68 days	After 142 days	At start	After 7 days	After 21 days	After 68 days	After 142 days
—	11	10	12	11	4	0	2	1	1	1	9	10	9	13	19
40°	7	9	10	8	3	0	1	3	1	1	9	11	15	14	32
56°	2	14	16	38	48	2	7	9	23	2	10	14	17	37	45
70°	2	17	11	24	27	3	8	11	16	20	12	16	23	22	27
100°	01	17	22	10	10	3	6	14	19	33	14	16	23	25	40

TABLE XV—*Continued.*

3. Various richer soils.

MT containing 16 % water, 0·26 % N, 1·0 % $CaCO_3$ and losing 7·8 % on ignition.
RC ,, 22 ,, 0·37 ,, 0·6 ,, ,, ,, 11·0 ,, ,,
Garden soil ,, 15 ,, 0·38 ,, 2·6 ,, ,, ,, 7·3 ,, ,,
KW ,, 12 ,, 0·18 ,, 0·4 ,, ,, ,, 7·6 ,, ,,

	Soil *MT*						Soil *RC*					
	N as NH_3, parts per million of dry soil			N as NH_3 and nitrate, parts per million			N as NH_3, parts per million of dry soil			N as NH_3 and nitrate parts per million		
	At start	After 32 days	After 114 days	At start	After 32 days	After 114 days	At start	After 28 days	After 112 days	At start	After 28 days	After 112 days
Untreated soil	4	8	9	50	63	66	24	12	10	188	178	241
Treated with toluene	5	44	35	49	100	93	39	103	132	196	255	298
Heated to 55°	10	5	6	62	67	78	49	93	9	185	292	270
,, ,, 100°	19	53	78	78	119	152	52	160	184	217	325	393

	Garden soil				Soil *KW*			
	N as NH_3, parts per million of dry soil		N as NH_3 and nitrate, parts per million		N as NH_3, parts per million of dry soil		N as NH_3 and nitrate, parts per million	
	At start	After 124 days	At start	After 124 days	At start	After 63 days	At start	After 63 days
Untreated soil	6	8	50	90	2	2	26	29
Treated with toluene	15	9	56	129	8	45	31	71
Heated to 55°	15	23	59	128	10	41	34	66
,, ,, 100°	22	87	69	165	11	45	34	66

TABLE XV—*Continued.*

4. Effect of drying.

(1) Soil *RC* containing 24 % water, composition when air-dried given above.

	Bacterial numbers, millions per gram			N as NH_3, parts per million			N as NH_3 and nitrate, parts per million			
	At start	After 26 days	After 42 days	At start	After 26 days	After 42 days	At start	After 26 days	After 42 days	Gain in 42 days
Untreated	27	28	39	5	2	3	91	105	105	14
Dried*	7	37	59	15	3	2	96	139	141	45
(2) Soil *KW*										
Untreated	5	5	8	0·5	2	1	57	66	65	8
Dried*	4	8	10	14	18	24	70	74	85	15

* For 10 days in a hot chamber at 35°—38°.

nitrate. But we know that this would have happened in any case as the result of the accumulation of ammonia and nitrate already formed and we cannot therefore conclude that it is connected with the drop in bacterial population. Indeed the decomposition has already gone so far before the reduction in bacterial numbers sets in—two-thirds of the final quantity of ammonia and nitrate being already formed, and the remaining one-third being on its way[1]—that but little work remains to be done.

§ 49. Entirely similar results were obtained in other experiments with arable soils, and in some of the experiments with richer soils. In (6) for instance (same Table), the bacterial numbers are at first much higher in the infected soils than in the toluened soil and the amount of decomposition subsequently becomes higher. But even after five months, when the process is well on to completion, there is no evidence that the fall in bacterial numbers has adversely affected the rate of accumulation of ammonia and nitrate, for the quantities actually found correspond fairly well with the numbers calculated on the assumption that the untreated soil and the toluened soil + water extract of untreated soil (*i.e.* toluened soil + bacteria of untreated soil but with minimum accompaniment of detrimental organisms) are inert to one another.

On the other hand, instances have also been observed where the drop in bacterial numbers is accompanied by a marked falling off in the rate of decomposition[2].

§ 50. The other experiments recorded in the Table illustrate the case where a large amount of ammonia, or of ammonia and nitrate, has already accumulated, and where fresh accumulation is no more rapid than in the untreated soils. No relationship therefore exists between bacterial numbers and rate of decomposition.

§ 51. The conclusion to be drawn from these experiments is that the increased bacterial numbers resulting from the introduction of the original flora into the partially sterilised soil leads to an increased production of ammonia and nitrate unless too large a quantity of these substances is already present. But the subsequent depression in bacterial numbers consequent on the development of the detrimental organisms is generally (though not always) without effect on the rate of decomposition, apparently because it does not set in until too late.

[1] We have shown in our previous paper that the amount of unstable intermediate products as well as of ammonia is increased by partial sterilisation.

[2] *E.g.* this *Journal*, 1912, **5**, 98.

The effect of varying temperatures of incubation on the changes taking place in partially sterilised soils.

§ 52. The experiments dealt with in the preceding sections were mainly carried out at the ordinary laboratory temperature. A series was now undertaken at higher temperatures, the bottles of soil being stored in incubators maintained respectively at 20°, 30°, 40° and 50°. The results obtained are set out in Table XVI.

We have already dealt with the bacteriological data and need now only point out that an increase in temperature from 10° to 20° fails to increase the bacterial numbers in the untreated soil, but it does increase them in the toluened soil, a fact that has been fully discussed in §§ 4 and 5. The bacterial numbers attain a maximum in the soil stored at 20° though they are still high at 30°, they fall off rapidly at 40° and still more completely at 50°. At these two higher temperatures there is no difference in bacterial numbers in the toluened and the untreated soils. The curves obtained in studying one of the soils are given in Fig. 7.

The rate of accumulation of ammonia and nitrate is connected with the bacterial numbers only in the toluened soils kept at 5°—12° and at 20°. The bacterial numbers increase at 20° in comparison with those at the lower temperature and so also does the rate of accumulation of ammonia and nitrate until the usual falling off sets in, when the relationship ceases to exist. In all other cases there is a complete absence of any sort of relationship. The rate of accumulation of ammonia and nitrate is greater at 20° than at 5°—12°, although the same limit may be reached in both cases; it is also generally greater in the toluened soil than in the untreated soil. At 30° it is still greater and in all but one instance it proceeds much further than at the lower temperatures; it becomes rapid at 40° and still more so at 50° and proceeds at each temperature to a correspondingly higher extent. We get, therefore, a series of curves in which the rate of accumulation of ammonia and nitrate successively increases with increasing temperature and in which also the extent of the accumulation also increases—to a small extent—at first but very much afterwards. This is wholly unlike the series obtained for bacterial numbers, excepting, as above mentioned, on the toluened soils at about 10° and 20°.

§ 53. Thus we have another exception to the rule that bacterial numbers are connected with the rate of accumulation of ammonia and

nitrates. But it is not necessarily a significant exception, for the decomposition at the higher temperatures may be a chemical process wholly unconnected with bacteria[1]. For the present we prefer to leave this

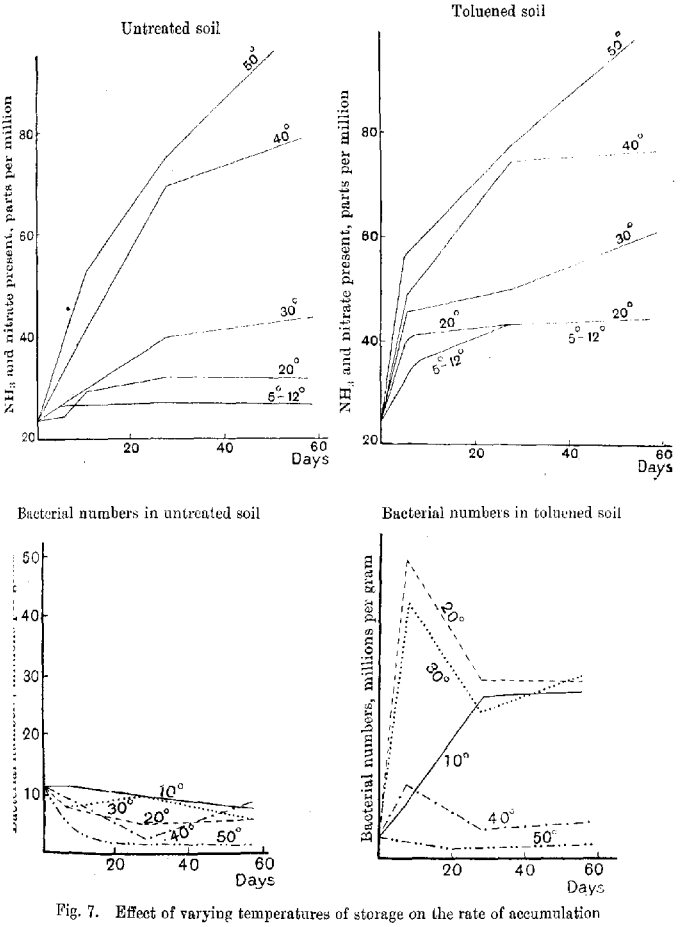


Fig. 7. Effect of varying temperatures of storage on the rate of accumulation of ammonia and nitrate in soils (Table XVI, arable soil).

[1] It will be noticed that ammonia accumulates in the soils maintained at 40° and over, showing that the nitrifying organisms no longer work much, if at all.

TABLE XVI. *Effect of varying temperatures on the changes taking place in partially sterilised soils.*

1. Arable soil as before containing 14 % water.

	Bacterial numbers, millions per gram			N as NH_3, parts per million			N as NH_3 and nitrate, etc., parts per million		
	After 5 days	After 27 days	After 58 days	After 5 days	After 27 days	After 58 days	After 5 days	After 27 days	After 58 days
Untreated soil kept at									
5°—12°	11	9	7	1	2	1	27	27	27
20°	8	5	6	2	2	1	24	32	31
30°	8	9	6	2	1	3	29	41	44
40°	9	2	8	2	13	36	43	70	79
50°	4	1	1	23	42	80	53	75	117
Toluened soil kept at									
5°—12°	8	27	28	13	19	23	32	44	45
20°	50	30	30	19	18	7	39	44	45
30°	43	24	31	23	15	3	46	50	62
40°	12	4	6	27	47	49	48	74	76
50°	2	1	1	35	56	72	56	78	102

TABLE XVI—*Continued.*

2. Rich Greenhouse soil *OxL* containing 40 % water, 0·63 % N, 1·9 % $CaCO_3$ and losing 17 % on ignition.

	Bacterial numbers, millions per gram					N as NH_3, parts per million					N as NH_3 and nitrate, etc., parts per million				
	At start	After 13 days	After 25 days	After 71 days	After 134 days	At start	After 13 days	After 25 days	After 71 days	After 134 days	At start	After 13 days	After 25 days	After 71 days	After 134 days
Untreated soil kept at															
5°—12°	65	63	41	32	46	15	25	22	18	11	372	391	390	441	326
20°	—	41	22	23	16	—	23	22	16	14	—	393	397	482	617
30°	—	27	51	16	19	—	20	22	18	9	—	402	452	643	833
40°	—	14	9	33	3	—	18	21	15	22	—	498	660	885	937
Toluened soil kept at															
5°—12°	9	73	101	138	108	81	94	101	197	161	428	439	438	543	470
20°	—	187	128	182	78	—	130	123	180	76	—	443	462	530	514
30°	—	197	145	51	20	—	181	193	14	15	—	529	532	476	636
40°	—	148	52	100	11	—	228	50	34	28	—	567	533	744	801

question undecided until other investigations now in hand of the chemical processes are further advanced. It may also be doubted whether our method of counting is applicable to the soils stored at higher temperatures. Gelatine plates kept at 20° may not provide suitable conditions for the development of such thermophilic organisms as may be active in a soil maintained at 40°.

§ 54. It is possible to treat the curves mathematically after the methods adopted by physical chemists, but the data are hardly fine enough since the error of the determinations becomes rather considerable at the higher temperatures. There is an unknown loss of ammonia (see footnote, p. 197) and there also appears to be a production of reducible substances not present in appreciable amounts in ordinary soils, but giving rise to ammonia on reduction with the zinc-copper couple, and therefore appearing in the analytical results as nitrates.

The effect of additional food on bacterial numbers and the rate of decomposition.

§ 55. It has already been pointed out that all the antiseptics used have some direct action in the soil which is shown in our experiments by a liberation of ammonia. We therefore have to consider the possibility that other substances may be set free capable of acting as food for bacteria, and it becomes necessary to ascertain how added foodstuffs affect the bacterial numbers and the amounts of decomposition. We have limited ourselves to three substances: sugar, hay dust and peptone.

§ 56. All of these substances cause the bacterial numbers to go up rapidly for a time, but there is no corresponding increase in the amounts of ammonia and nitrate such as is obtained on partial sterilisation. Sugar, indeed, causes a marked loss of nitrate and no increase in ammonia; hay dust has a similar but less marked action, whilst peptone fails to increase the stock of ammonia and nitrate even by the full amount of the nitrogen it contains. Supposing therefore any foodstuff to be liberated by the antiseptics used we should expect it to increase the bacterial numbers but not necessarily the ammonia and nitrate. This would happen only if the liberated substances themselves gave rise to the extra ammonia and nitrate, *i.e.* if they were easily decomposable after contact with antiseptic vapours, but not before.

The limitations of the methods for counting bacteria.

§ 57. Three facts stand out prominently on reviewing the whole of the data obtained at ordinary temperatures (*i.e.* not above 20°):

(1) An increase in bacterial numbers effected by partial sterilisation commonly causes an increase in the amounts of ammonia and nitrate.

(2) But if a large amount of ammonia or of ammonia and nitrate is already present in the soil the increased bacterial numbers do not necessarily bring about more production of these substances.

(3) Whenever an increase in the rate of production of ammonia and nitrate is obtained there is always an increase in bacterial numbers[1].

(2) and (3) may also be expressed thus: bacterial multiplication may take place without an increased rate of production of ammonia and nitrate, but an increased rate of production of ammonia and nitrate does not occur without bacterial multiplication.

§ 58. In some of our experiments the relation between bacterial numbers and amounts of ammonia and nitrate is fairly close (*e.g.* Fig. 4, Case 1), but more usually this is not so. From what we know of the limitations of the counting method indeed, we should hardly expect it to be otherwise. Some of the soil bacteria are more vigorous ammonia producers than others, some are not even active but only occur as spores. Yet all these are grouped together without any distinction. Further, the method does not even give the actual total but misses altogether those organisms that fail to develop on the plates employed; there is, in fact, no method for estimating the total number of bacteria in the soil.

§ 59. The question therefore arises, how far do the numbers revealed by any particular plate possess any significance? The data for a complete answer do not exist, but there is considerable evidence that on one and the same soil under strictly comparable conditions the fluctuations in the numbers growing on gelatine plates afford a satisfactory index of the fluctuations in the total numbers of decomposition bacteria. When therefore we find that partial sterilisation has increased the bacterial numbers revealed by our plates from 20 to 40 millions per gram we do not imply that the true totals have doubled under the treatment, but that they have increased; the exact amount of increase we cannot as yet specify. Further, we only institute the comparison where the conditions are otherwise identical; the untreated

[1] In the course of four years we have only found one exception. The results obtained at 30°, 40° and 50° are expressly excluded here.

TABLE XVII. *Effect of added organic matter on the bacterial numbers and rate of decomposition in untreated and partially sterilised soils.*

A. Arable soil containing 15 % moisture, 0·18 % N, 0·37 % $CaCO_3$ and losing 7·6 % on ignition.

	Bacterial numbers, millions per gram				N as NH_3, parts per million of soil				N as NH_3 and nitrate, parts per million of soil			
	After 6 days	After 62 days	After 101 days	After 215 days	After 6 days	After 62 days	After 101 days	After 215 days	After 6 days	After 62 days	After 101 days	After 215 days
Untreated soil alone	13	7	11	12	4	2	4	1	(57)	71	85	
,, ,, +0·25 % sugar	129	97	32	26	2	3	2	6	5	24	37	98
,, ,, +0·1 % peptone*	148	11	21	8	60	45	28	31	117	172	177	71
,, ,, +hay infusion	19	7	9	7	3	2	4	5	56	85	91	231
												106
Toluened soil alone	19	3	27	23	16	31	30	54	58	79	80	
,, ,, +0·25 % sugar	19	11	33	28	5	36	35	59	16	42	42	101
,, ,, +0·1 % peptone*	14	19†	44	32	41	194†	120	158	89	227†	169	63
,, ,, +hay infusion	15	8	22	24	10	32	41	47	54	77	95	204
												95
Toluened soil +5 % untreated soil alone	118	45	48	lost	8	31	26	37	56	79	84	
Toluened soil +5 % untreated soil +0·25 % sugar	156	133	134	77	2	32	28	31	7	39	46	104
Toluened soil +5 % untreated soil +0·1 % peptone*	125	102	102	58	50	109	113	116	97	157	173	67
Toluened soil +5 % untreated soil +hay infusion	114	66	61	17	10	29	27	25	56	82	89	210
												94
Toluened soil +5 % rich greenhouse soil untreated alone	84	25	106	lost	13	12	4	0	66	89	103	
Toluened soil +5 % rich greenhouse soil untreated +0·25 % sugar	178	100	163	59	4	12	12	3	12	57	74	123
Toluened soil +5 % rich greenhouse soil untreated +0·1 % peptone*	82	80	77	35	84	54	58	40	144	192	212	92
Toluened soil +5 % rich greenhouse soil untreated +hay infusion	130	75	51	13	35	9	10	6	93	94	110	243
												118
At beginning of experiment Untreated soil	8				4				57			
,, ,, ,, Toluened soil	5				6				54			

TABLE XVII—*Continued.*

B. Arable soil containing 15 % moisture, 0·18 % N, 3·16 % $CaCO_3$ and losing 4·6 % on ignition, organic matter added = 0·5 % ground hay containing 1·43 % N and adding 71·5 parts of N per million of soil.

	Bacterial numbers, millions per gram			N as NH_3, parts per million			N as NH_3 and nitrate, parts per million		
	At start*	After 7 days	After 74 days	At start*	After 7 days	After 74 days	At start*	After 7 days	After 74 days
Untreated soil, no hay	7	4	12	3	2	1	27	25	27
,, ,, + hay	7	94	62	3	3	4	27	13	12
Toluened soil, no hay	31	41	38	25	47	4	43	67	58
,, ,, + hay	31	175	136	25	12	10	43	23	20
Toluened soil + water extract of untreated soil, no hay	50	—	56	2	2	2	58	66	67
,, ,, ,, ,, ,, + hay	50	311	152	2	2	3	58	45	45
Toluened soil + 0·5 % untreated soil, no hay	66	43	65	3	2	2	59	63	65
,, ,, ,, ,, + hay	66	227	101	3	3	2	59	46	50
Toluened soil + 5 % untreated soil, no hay	57	58	47	2	7	3	64	67	66
,, ,, ,, ,, + hay	57	255	83	2	3	3	64	44	47
Toluened soil + 50 % untreated soil, no hay	25	—	53	2	—	2	46	—	49
,, ,, ,, ,, + hay	25	86	66	2	3	4	46	25	27

* *I.e.* when hay was added; the soils had been treated 5½ months previously in order that the initial changes in the soil itself might be finished before the hay was put in.

and partially sterilised soils were initially the same, the water content, temperature and aeration conditions are as nearly as possible identical; no attempt is made to compare one soil with another under different conditions. With these limitations the method gives valuable results and can continue to be used till a better is devised.

Other media are in use besides gelatine, but so far as they have been tried they give the same kind of results, indicating fluctuations in the same direction as gelatine although the actual figures are different.

§ 60. Perhaps the most striking proof of the validity of the gelatine plate method as we use it is that it leads to precisely the same conclusions as methods of a wholly different character. For example, the gelatine plate method shows that the numbers of bacteria increase after partial sterilisation. We have seen that special tests of an entirely different nature show an increase of denitrifying organisms and of organisms causing loss of nitrogen after partial sterilisation (footnote, p. 178); in our previous paper we showed that the rate of decomposition of peptone solution (in Remy's method) also increases. We have used these peptone solutions in testing some of the deductions drawn from the gelatine plate experiments, and have invariably found that both methods gave the same results. Two instances only need be given:

(1) From the gelatine plate counts we concluded that the effect of partial sterilisation was to improve the soil as a medium for bacterial growth and not to improve the bacteria as decomposition agents (§§ 24 and 25). When *small* inoculations of soil are made into peptone solutions so that the effect shall be that of bacteria and not of soil, the partially sterilised soil is no better than the untreated. But when *large* inoculations are used, so that the effects of the detrimental organisms can come into play, the partially sterilised soil is distinctly better than the untreated. The amounts of ammonia in milligrams produced from a one per cent. peptone solution were:

		Hours			
	After	24	32	44	90
Small inoculation (effects of bacteria only)	Untreated soil.....	0·5	7·3	8·7	21·0
	Toluened soil	0·5	7·5	8·5	20·9
Large inoculation (effects of bacteria and of detrimental organisms)	Untreated soil.....	4·9	15·2	21·6	33·5
	Toluened soil	6·2	18·0	23·6	34·5

(2) From the gelatine plate experiments we concluded that the detrimental factor was not associated with the water extract but with

the soil itself. A *clear, filtered* water extract of the untreated soil was found to be more effective in decomposing peptone than a similar extract of toluened soil, but a *turbid* extract was distinctly less effective. The amounts of ammonia in milligrams produced from one per cent. peptone were:

	Hours	
After	65	90
Suspension of untreated soil, muddy	20·4	28·4
,, toluened ,, ,,	24·2	34·7
,, untreated soil filtered through cotton wool, turbid	19·1	25·1
,, toluened ,, ,, ,, ,, ,,	20·1	30·0
,, untreated soil filtered through paper, clear	5·0	11·2
,, toluened ,, ,, ,, ,,	5·9	8·7

Thus in both cases the experiments lead to the same conclusion although the methods are fundamentally different. On the whole we prefer the gelatine plates to the culture solutions, but we use the culture solution methods to check the results yielded by the gelatine plates.

Summary and Conclusions.

The conclusions reached in our previous paper have been confirmed and extended. Fresh evidence is adduced that bacteria are not the only inhabitants of the soil, but that another group of organisms occurs, detrimental to bacteria, multiplying more slowly under soil conditions and possessing lower power of resistance to heat and to antiseptics.

In consequence of the presence of these detrimental organisms the number of bacteria present in the soil at any time is not a simple function of the temperature, moisture content and other conditions of the soil. It may, indeed, show no sort of connection with them; thus rise of temperature is found to be ineffective in increasing the bacteria in the soil; increase in moisture content has also proved without action. The number of bacteria depends on the difference in activity of the bacteria and the detrimental organisms.

But when soil has been partially sterilised the detrimental organisms are killed and the bacteria alone are left. It is then found that increase in temperature (up to a certain point) favours bacterial multiplication and causes the numbers to rise. Variations in moisture content also produce the normal results on partially sterilised, but not on untreated soils.

The detrimental organisms are killed by any antiseptic vapour or by heating the soil to 55°—60° C.: they suffer considerably when the

soil is maintained at lower temperatures (40° C.) for a sufficient length of time. Cooling to low temperatures also depresses them although it fails to kill them.

The completeness of the process can be accurately gauged by the extent to which the bacteria suffer. Whenever the treatment is sufficiently drastic to kill the nitrifying organisms and to reduce considerably the numbers of the other bacteria (as shown by the counts on gelatine plates) it also kills the detrimental organisms. If the soil conditions are now made normal, and the antiseptic is completely removed, rapid increase is observed in the bacterial numbers and the rate of production of ammonia. A temporary or partial suppression of the factor is, however, possible without extermination of the nitrifying organisms.

Once the detrimental organisms are killed the only way of introducing them again is to add some of the untreated soil. But the extent of the transmission is apt to be erratic, being sometimes more and sometimes less complete than at others; occasionally the infection fails altogether. We have not yet learned the precise conditions governing the transmission.

Provisionally we identify the detrimental organisms with the active protozoa of the soil, but as the zoological survey is yet incomplete we do not commit ourselves to any particular organism or set of organisms or to any rigid and exclusive definition of the term protozoa.

The increase in bacterial numbers following after partial sterilisation by volatile antiseptics is accompanied by an increase in the rate of ammonia production until a certain amount of ammonia or of ammonia and nitrate has accumulated, when the rate falls. Thus two cases arise: (1) when only small amounts of ammonia and nitrate are present there is a relationship between bacterial numbers and the rate of ammonia production, (2) when large amounts of ammonia or of ammonia and nitrate are present there is no relationship. The limit varies with the composition and condition of the soil.

Complications are introduced when the soil has been partially sterilised by heat, because heat effects an obvious decomposition of the organic matter, thus changing the soil as a medium for the growth of micro-organisms. The bacterial flora is also very considerably simplified through the extermination of some of the varieties. These effects become more and more pronounced as the temperature increases, and their tendency is to reduce the numbers of bacteria. We find maximum bacterial numbers in soils that have been heated to the minimum

temperature necessary to kill the detrimental organisms (about 60°). Both bacterial numbers and the rate of decomposition in such soils approximate to those obtaining in soils treated with volatile antiseptics, and the above-mentioned relationships between these quantities also hold.

Although bacterial numbers are at a minimum in soils heated to 100° the decomposition effected is at a maximum.

With this exception it is generally true that bacterial multiplication may go on without increasing the rate of production of ammonia, but an increase in the rate of production of ammonia does not take place without bacterial multiplication.

The increase in bacterial numbers brought about by addition of bacteria from the untreated soil into partially sterilised soil leads to still further production of ammonia and nitrate unless too large a quantity of these substances is already present. But the subsequent depression in bacterial numbers consequent on the development of the detrimental organisms is generally (though not always) without effect on the rate of decomposition, apparently because it does not set in until too late.

THE MICRO-FLORA OF STILTON CHEESE.

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When well made a Stilton is usually admitted to be the finest of the English varieties of cheese, but like all choice dairy products it is exceedingly difficult to manufacture of uniformly good quality. Great uncertainty exists, even amongst the most experienced makers, in regard to the factors which govern the excellence of flavour and texture in this kind of cheese: the high moisture content favours the rapid growth of harmful as well as useful organisms and "off flavour," objectionable taints, and irregular consistence due to the activity of the former class are common.

Some five years ago the authors began the study of the micro-flora of Stilton cheese, in the hope that a more complete knowledge of the organisms present would assist in the elucidation of the ripening process, and be a step towards placing the manufacture of cheese of fine quality on a sound basis.

In the first instance cheeses made at the British Dairy Institute under the supervision of Mr Miles Benson were investigated; later examples from the best dairies of the Melton Mowbray district were chosen for examination. Our work has been directed towards the determination of the kinds of bacteria and fungi present in normally ripened cheeses: at the same time we have endeavoured to estimate the numbers of organisms in cheeses at different periods of ripening. The investigation of both these problems has proved of great complexity, and much remains to be done.

We have, however, thought it opportune to give an account of the work already accomplished, for in regard to the normal aerobic species of bacteria and fungi which are found in this variety of cheese we consider that the investigation is fairly complete. We have not attempted to deal with the parts which the several kinds of organisms play in the development of aroma, flavour, and texture of the cheese;

these problems, as well as the search for anaerobic organisms, must be left for future study.

Attention was first paid to a determination of the number of organisms in the cheese at various periods, from 24 hours old, up to *complete ripeness, 80—100 days later.*

Cylindrical pieces were withdrawn with a sterilised cheese-borer, driven into the cheese to approximately the same depth each time: one gram of the extracted core was rapidly weighed, and then rubbed up in water in a small sterilised glass mortar.

This was ultimately diluted to 1 in 1,000,000, and 1 c.c. of the dilution was added to a tube of the medium used for plating out. It was found that dilution to 1 in 100,000 was insufficient to allow of precise counting of the colonies, especially where very young cheeses were under examination.

Three media were used, namely lactose-gelatine (100 grams gelatine and 20 grams lactose per litre of medium, acidity 10+), a similarly acid lactose agar (1·5 % agar) and a neutral litmus lactose-gelatine.

After incubation of the gelatine plates at 22 deg. C. and the agar at 35 deg. C. for two days, the colonies were counted.

The distribution of the organisms in the cheese was studied by cutting thin slices of the latter with a razor and staining with eosin-methylene blue or thionine: examinations were also made of cover-glass impressions of the broken curd: both methods revealed an uneven grouping of organisms. Single isolated bacteria, mainly rods, occur fairly evenly distributed throughout the cheese, but the majority are found in irregularly arranged colonies of very variable size. On account of this irregularity in the disposition of the organisms, it is not possible to obtain strictly comparable samples from day to day from the same cheese, without taking a large number of separate borings on each occasion, a proceeding which would render the cheese useless for future investigation.

The results of our work indicate that so far as an estimation of the number is concerned, a gram of cheese taken from a single boring is too small a portion to be regarded as completely representative of the bulk of the core withdrawn. Moreover the numbers obtained differ with different media used for plating out: some grow better on one substance than on another, the numbers being considerably less on agar than on either of the gelatines employed. As indicated later, the presence of one kind of organisms more or less entirely prevents

the appearance of another species on the same plate. Extended trials have shown that a complete and precise estimate of the numbers of organisms in a cheese of the type of Stilton with its very moist and loosely packed curd cannot be attained by small samples obtained from single borings. However, repeated examination during the last four or five seasons of portions of single borings removed from the cheeses at short intervals has enabled us to obtain a general view of the rise and fall in the numbers of organisms and the changes which occur in the micro-flora as ripening proceeds.

In the first 48 hours there is an extraordinary development of organisms from 1000 to 3000 million per gram being frequently found, more than 90 $^{0}/_{0}$ of which are cocci or short rods capable of producing lactic acid in milk. The numbers reach a maximum within four days, and decline slowly afterwards up to the time of complete ripeness, when the cheese contains from 50 to 100 millions per gram, mostly moulds and yeasts; concurrent with the diminution of bacteria there is a gradual rise in the development of fungi.

As mentioned later, a considerable number of species of bacteria and fungi are found in Stilton cheese, the greatest variety being found on plates from young specimens before the acidity has reached its maximum, and in the later periods of ripening when the acidity is lowest and open spaces due to shrinkage have begun to form: occasional moulds are met with throughout the whole period of ripening, but they make their appearance in increasing numbers when the cheese is 30—40 days old.

The great variety of organisms we have isolated may be classified into two groups, namely:—

(1) Bacteria and fungi, which from their constant occurrence in Stilton cheeses whenever and wherever they are made, may be regarded as normal constituents of the micro-flora, and concerned more or less directly with the ripening process of a good cheese.

(2) A miscellaneous group whose presence is accidental and whose influence is either harmless or detrimental to the quality.

Normal Bacteria. Three forms of bacteria are invariably present, namely, a coccus, a rod-shaped organism belonging to the lactic acid group, and a species of *Tyrothrix*.

1. *Coccus A* is a small roundish-oval organism, 0·4—0·5 μ in diameter (Fig. 1). It usually occurs in pairs, but occasionally may be found in short chains of three and four together. It grows slowly on solid media in the form of minute white colonies beneath the surface.

In milk it produces a dense uniform curd without gas bubbles or separation of whey. It curdles milk slowly, taking three days at 22 deg. C. to produce a solid curd.

We consider this typical *Streptococcus lacticus*. It is abundant in all cheeses we have examined.

A larger form is found in some cheeses with oval cells somewhat elongated—1·1 μ to 1·3 μ long. Just before or at the time of division, the organism resembles a short rod. In milk and in cheese 24 to 48 hours old it is found in well-developed chains, consisting of 6 to 12 or more cells. In cheeses a few days old, however, and on gelatine and agar media, the chain form is lost, the organism then appearing chiefly in pairs (Figs. 2 and 3). The colonies are white and very small —$\frac{1}{2}$ mm. or less in diameter: they grow beneath the surface of the medium. Milk is curdled by this bacterium in 24 hours or less when kept at 22 deg. C., the curd produced being dense and uniform, without gas bubbles or separation of whey.

The organism is a form of *Streptococcus lacticus*, and appears to be identical with the coccus present in most of the commercial starters so commonly used by butter makers and some makers of cheese: Stiltons containing it are of fair mild flavour, but too dry and acid. Possibly vigorous acid formers like this species may be useful in repressing the objectionable organisms found in dirty dairies, or where cheese is manufactured from doubtfully clean milk; we feel convinced, however, that they should find no place in a Stilton dairy where cheeses of the finest flavour and texture are desired.

2. *Bacterium A*. Another common characteristic bacterium in Stilton cheese is a short stumpy rod, 2—4 μ long and 0·7—0·8 μ thick (Fig. 4): sometimes it occurs in the form of slightly oval cells, and then resembles the large *Streptococcus* referred to above.

The colonies on the surface of the media are circular, from 1—2 mm. in diameter, white, moist and raised a little above the surface; in the substratum they are smaller and either round or spindle-shaped; old colonies become yellowish.

The organism is Gram positive and non-motile.

It curdles milk slowly, taking 6—8 days at 22 deg. C. to produce a dense curd, which very closely resembles that thrown down by *Streptococcus lacticus*; no gas bubbles appear, and there is little or no separation of whey.

When grown in broth it lengthens to 4 or 4·5 μ, but preserves its somewhat oval shape: it appears to be a variety of *Bs. acidi lactici*

of Hueppe, but unlike the latter it does not ferment glucose nor lactose: some forms of it give a slight Voges and Proskauer reaction, but none produce indole.

In fully ripe cheeses lactic acid organisms are comparatively few in number; many die off altogether in the ripening process, and those which remain possess diminished vitality and are only able to acidify milk very slowly.

3. A species of *Tyrothrix* is invariably present in Stilton cheeses. It is found in all stages of ripening after the third or fourth day, but is never abundant.

It is the chief and usually the only organism which appears on plates inoculated with a "dilution" of the cheese heated to boiling point. The organism is rod-shaped, and feebly motile in young cultures. The individual cells from agar colonies are 6—12 μ long, 1—1·25 μ broad. In milk they are longer and thinner and often united in the form of tangled threads, some of which may reach a length of 150 μ (Fig. 5).

It forms oval spores, which measure 2 $\mu \times 1$ μ.

Gelatine is liquefied rapidly by it, and milk is rendered alkaline and coagulated, the curd being soon digested: no gas is produced in glucose, saccharose, or lactose bouillon.

The surface colonies on agar are white, round, smooth and moist at first; later they spread over large areas and become wrinkled and dry, the margins of such colonies being fimbriated. Beneath the surface of the medium the colonies remain small and are more or less granular with characteristic mycelioid or floccose margins.

The organism is closely allied to, if not identical with, Duclaux's *Tyrothrix tenuis*.

4. *Miscellaneous Bacteria.* In addition to the four species of bacteria already mentioned, which we consider from their physiological activities and constant presence are directly concerned with the ripening of Stilton cheese, there are many others of accidental occurrence. In the latter class we include *Bacterium coli*, *B. lactis aerogenes*, and many chromogenic species of bacilli and cocci giving rise to violet, pink, yellow and orange colours.

The number of kinds are found to vary with the season when the cheese is made and the source from which the milk is derived. Of this miscellaneous group, *B. lactis aerogenes* is the most commonly found: typical *B. coli* is rare.

Fungi. In the course of our investigations we have observed a

considerable number of species of fungi, those of most frequent occurrence being *Oospora lactis*, *Mucor mucedo*, *Aspergillus glaucus*, *Cladosporium herbarum*, *Penicillium glaucum*, and several forms of *Torula*. The four first mentioned appear only on the coat of the cheese, rarely or never in the interior. They may be regarded as accidental or unavoidable in the ordinary course of manufacture of Stilton cheese, and apparently play little or no part in the ripening process.

Oospora is very abundant on the outside during the first 15 to 20 days, when the coat of the cheese is moist.

Mucor, *Aspergillus*, and *Cladosporium* are more casual in their occurrence, and may appear upon cheese of all ages in small irregular numbers.

The fungi which are undoubtedly of great importance in the ripening process of Stilton cheeses are *Penicillium glaucum* and one or two forms of *Torula*; these are found throughout the interior of the cheese.

When cut across a well-made ripe Stilton exhibits a characteristic mottling of blue veins most abundant in the softer centre, and radiating in an irregular manner towards the firmer outside. These veins are crevices more or less filled with the mycelium and conidiophores of *Penicillium glaucum*. We have observed conidia in small numbers in the fresh curd; they are, however, very sparsely distributed and difficult to find, and the fungus rarely occurs on plates inoculated from cheeses less than three weeks old. Either from want of air or from the presence of inhibiting substances the spores appear to be prevented from germinating in the closely packed curd in which they are imbedded. In the shrinking cheese cavities arise at the points where the separate pieces of curd first packed in the mould touch each other: it is in these cavities that the mycelium of the fungus makes its appearance and spreads over the surface of the crevices at a rapid rate without penetrating into the substance of the cheese more than a very small fraction of an inch.

The mycelium is colourless, and it is not till the formation of the conidiophores and the conidia that the blue-green tint of the "veins" in the cheese is developed.

In open spaces with plenty of room for growth the conidiophores are of the ordinary type, the hyphae being about 3 μ in diameter, with sterigmata 5·5—8 μ long, bearing round smooth conidia 2·8—4 μ in diameter. When seen in mass the conidia are of a bluish-green tint:

grown on agar or gelatine media the blue-green tint of the colonies ultimately changes to a greyish brown or mouse colour.

We found that in many of the crevices, especially those of small dimensions, the hyphae of the mycelium become much thickened, their diameter reaching 9—12 μ. The apex of such thickened hyphae may give rise to a much thinner hypha which develops into a conidiophore (Fig. 7 *b*). Sometimes sterigmata and conidia arise on a very short hypha placed laterally upon the thick mycelial hypha as in Fig. 7 *a*. Not infrequently we have observed the development of a single sterigma with its conidia within the individual cells formed by partitions across the thickened hypha: the protoplasm of the cell shrinks, leaving an enclosed space, into which the fructification develops later (Fig. 7, *c* and *d*), such remarkable phenomena not only occurring at the apex of a hypha but sometimes in cells along the hyphae at random. Ascocarps have not been found.

We find that *Penicillium glaucum* does not grow on agar lactose media when the latter is inoculated at the same time with the Stilton *Tyrothrix*, although it grows luxuriantly enough in the presence of lactic acid organisms. The conidia germinate but the hyphae produced remain very short where *Tyrothrix* is growing freely; later, after the formation of spores by the *Tyrothrix*, the fungus develops rapidly.

We hope to further study the influence of these organisms upon each other.

Yeasts. One of the most frequent constituents of the micro-flora of Stilton cheese is a species of *Torula*. It occurs in cheeses of all ages, being abundant in those 24 hours old, as well as in those which are completely ripe.

The fungus is not only found on the coat, but is distributed throughout the interior of the cheese.

Plates inoculated with dilutions prepared from ripe cheeses exhibit colonies of the *Torula* in abundance, along with those of *Penicillium* and a few bacteria.

The cells of the Stilton *Torula* are round, 3·5—5 μ in diameter (Fig. 6).

Growth takes place more freely upon acid media than upon alkaline substrata. The colonies are round, white, and opaque, with smooth shining surfaces.

Less frequently met with is another form of yeast-like fungus, with oval cells 5·5—6 μ long and 3·5—4 μ broad. The colonies are white and opaque, with dull matt surfaces, which ultimately become wrinkled.

Fig. 1.

Fig. 5.

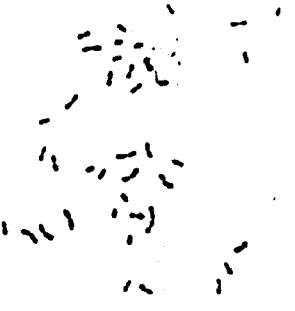


Fig. 2.

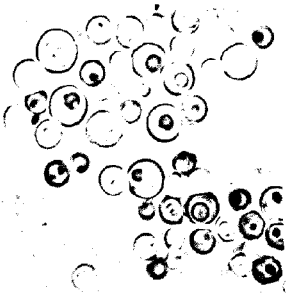
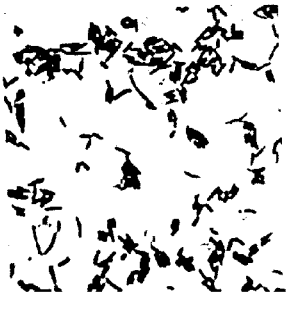
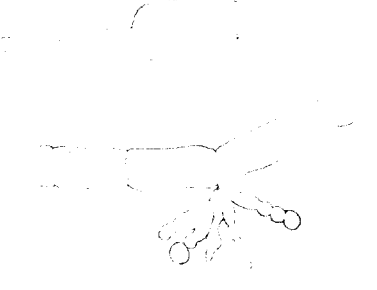


Fig. 6.

Fig. 3.





We have not been able to induce the formation of endospores in either of these "yeasts."

Summary.

1. The numbers of bacteria and fungi in a newly-made Stilton may rise to the enormous number of 1000 to 3000 millions per gram in the first week.

2. There is a gradual fall in the numbers up to the time of ripeness (100 to 150 days old), when 50 to 100 millions only are found.

3. In the early stages lactic acid bacteria are most abundant. When the cheese is ripe the lactic acid bacteria are few and weakened in physiological power; *Penicillium glaucum* and a form of *Torula* are then abundant.

4. Five characteristic organisms are found in all Stilton cheeses examined, viz.:—(1) *Streptococcus lacticus*, (2) a short rod-shaped form of *Bs. acidi lactici*, (3) a species of *Tyrothrix*, (4) *Penicillium glaucum*, and (5) a round form of *Torula*, sometimes accompanied or replaced by an oval form.

In cheeses where starters have been used we find a large celled form of *Streptococcus lacticus* present.

5. *Penicillium glaucum* is checked in its growth by the *Tyrothrix*.

We wish to acknowledge our indebtedness in this investigation to a grant made from the Development Fund by the Board of Agriculture and Fisheries.

EXPLANATION OF FIGURES IN PLATE VI.

The photomicrographs were taken with a 2 mm. Zeiss apochromatic objective and No. 8 eye-piece by Mr F. O. Mosley, University College, Reading.

Fig. 1. Small common form of *Streptococcus lacticus* from agar, × 900.
,, 2. Large form of *Streptococcus lacticus* from agar, × 900.
,, 3. ,, ,, ,, ,, milk, × 900.
,, 4. Short-rod lactic organism from agar (*Bs. acidi lactici* Hueppe), × 900.
,, 5. Stilton cheese *Tyrothrix* from agar, × 900.
,, 6. *Torula* from Stilton cheese from agar culture, × 900.
,, 7. Abnormal forms of hyphæ and conidiophores of *Penicillium glaucum* from interior of Stilton cheese.